Glutamate Dysfunction In First Episode Psychosis and Relationship With The Response To Treatment

Merritt, Kate Sky

Awarding institution:
King's College London

GLUTAMATE DYSFUNCTION IN FIRST EPISODE PSYCHOSIS AND RELATIONSHIP WITH THE RESPONSE TO TREATMENT

Kate Merritt

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Abstract

Abnormal glutamatergic neurotransmission is strongly implicated in the pathophysiology of schizophrenia. The main technique available for assessing central glutamate function in man *in vivo* is proton magnetic resonance spectroscopy (1H-MRS), which can be used to measure glutamate, its metabolite glutamine, or their combination (Glx). Although around sixty 1H-MRS studies in schizophrenia have been published, the findings have been inconsistent, and the extent to which these vary with the brain region examined, the stage of the disorder, the severity of symptoms and the effects of treatment is unclear. Nevertheless, data from recent cross-sectional studies suggest that glutamate concentrations may relate to the degree to which patients respond to antipsychotic medication. However, it is not yet known whether glutamate is predictive of the future therapeutic response, or whether glutamate concentrations change as a consequence of treatment. This issue can be addressed through the longitudinal assessment of glutamate concentrations in patients with psychosis before and after antipsychotic treatment.

A meta-analysis of the entire literature to date indicates that schizophrenia is associated with elevated 1H-MRS glutamate metabolites in the medial temporal cortex, basal ganglia, and thalamus, and that these findings vary with the stage of the disorder.

The relationship between 1H-MRS glutamate metabolites and symptom severity was examined in a large dataset of individual patient data, pooled from multiple research samples. However this did not identify robust associations between glutamate measures and symptom scores, consistent with the findings from a systematic review of studies that had examined this issue.

To investigate whether glutamatergic differences between antipsychotic responders and non-responders are predictive or consequential to the therapeutic response, a longitudinal (10 month) 1H-MRS study in FEP was conducted. This revealed that Glx levels in the thalamus declined with antipsychotic treatment in patients who responded well, but did not change in patients with a poor response after both 5 weeks and 10 months. Parallel work involving repeated 1H-MRS scanning of healthy volunteers indicated that these findings were not attributable to non-specific time effects.

Overall, the results from this thesis suggest that alterations in glutamatergic function are evident in a number of brain regions in schizophrenia, and that these differ between patients who do and do not respond to treatment with antipsychotic medication. These

findings have implications for our understanding of the pathophysiology of the disorder, the stratification of patients, and the development of novel treatments.

List of Abbreviations

ACC – Anterior Cingulate Cortex

ANOVA – Analysis of Covariance

BG – Basal Ganglia

BRPS – Brief Psychiatric Rating Scale

Cr – Creatine

CRLB – Cramer-Rao Lower Bounds

CSF – Cerebrospinal Fluid

DA – Dopamine

DLPFC – Dorsolateral Prefrontal Cortex

GABA – Gamma-Amino-Butyric-Acid

Gln – Glutamine

Glu – Glutamate

Glx – Glutamate and Glutamine signals combined

FEP – First Episode Psychosis

HR – High Risk

mPFC – Medial Prefrontal Cortex

MRI – Magnetic Resonance Imaging

1H-MRS – Proton Magnetic Resonance Spectroscopy

ms – milliseconds

MTL – Medial Temporal Lobe

NAA – N-Acetyl-Aspartate

NMDA – N-Methyl-D-Aspartate

PANSS – Positive and Negative Symptom Scale

PCP – Phencyclidine

PET – Positron Emission Tomography

PRESS – Point Resolved Spectroscopy

SD – Standard Deviation

SE – Standard Error

STEAM – Stimulated Echo Acquisition Mode

SZ - Schizophrenia

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Personal Contributions

I was involved in the implementation, analysis and writing up of all studies described in this thesis.

CHAPTER 1 - Introduction

1.1. Schizophrenia

Schizophrenia is a common, severe mental health disorder which affects 0.7% of the world's population (Saha et al., 2005). Schizophrenia is one of the leading causes of adult disease burden; it greatly affects an individual's quality of life and increases the risk of premature mortality (Whiteford et al., 2015). In addition to the personal costs of schizophrenia, it also incurs large costs to the economy, costing £11.8 billion per year in England (Elert, 2014).

Schizophrenia is characterised by the presence of psychotic symptoms such as delusions and hallucinations, negative symptoms such as apathy and social withdrawal, and cognitive deficits such as poor working memory and executive function. Schizophrenia emerges during late adolescence, with the average age of onset being 26 years in males and 30 years in females, with a second period of onset in females around the time of menopause (Häfner et al., 1993). Lifetime risk does not differ between genders, although a greater proportion of males are diagnosed from adolescence to around 35 years old (Häfner, 2003).

In 1952 the first antipsychotic medication, chlorpromazine, was discovered. Chlorpromazine was identified as a D2 receptor antagonist twenty years later, leading to the dopamine hypothesis of schizophrenia (Carlsson, 1977; I. Creese et al., 1976; Matthysse, 1973; Seeman and Lee, 1975). At present, all FDA-approved antipsychotic drugs act at the D2 receptor (Elert, 2014). Despite the success of dopamine antagonism as the mainstay treatment of schizophrenia, one third of patients do not respond to current medicines (Lehman et al., 2004), and so it has been proposed that other neurotransmitter systems may also play a role in schizophrenia aetiology.

1.1.1. The Dopamine hypothesis of Schizophrenia

The dopamine hypothesis of schizophrenia proposes that psychotic symptoms result from excessive dopaminergic signalling in the striatum (Carlsson and Lindqvist, 1963). The mechanism of action of antipsychotic drugs formed the basis of the dopamine hypothesis, as all antipsychotic drugs target the D2 receptor (Elert, 2014), and the potency of D2 antagonism strongly correlates with the dosage needed for clinical efficacy (I Creese et al., 1976; Seeman and Lee, 1975). Furthermore, dopamine enhancing drugs such as amphetamine possess psychotomimetic properties (Lieberman et al., 1987). Firstly, repeated exposure to amphetamine in healthy individuals can induce psychosis, with a

single exposure producing transient positive symptoms, predominantly those of paranoid ideation and auditory hallucinations (Angrist and Gershon, 1970; Bell, 1973). Secondly, low doses of amphetamine exacerbate psychotic symptoms in patients with schizophrenia (Lieberman et al., 1987).

PET studies have confirmed increased dopamine synthesis and release in patients *in vivo* (Howes et al., 2012a). The former is evidenced by increased uptake and storage of fluorine 18–labelled dopamine in the striatum, which corresponds to increased activity of the enzyme dopa-decarboxylase which is involved in dopamine synthesis (Dao-Castellana et al., 1997; Hietala et al., 1999, 1995; Lindström et al., 1999; McGowan et al., 2004; Meyer-Lindenberg et al., 2002; Reith et al., 1994). Increased striatal dopamine release in patients with schizophrenia is evident following amphetamine challenge (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996) and following dopamine depletion in patients (Abi-Dargham et al., 2000; Lawrence S Kegeles et al., 2010). The extent of increased dopamine release depends on illness stage; emerging during the prodromal phase (Howes et al., 2009) and being most prominent during the onset of illness and in patients experiencing an exacerbation of disease, rather than those in remission (Laruelle et al., 1999). Furthermore the degree of striatal dopamine release is correlated with the worsening of positive symptoms (Laruelle et al., 1999). These effects have been localised to the associative striatum, rather than the ventral or sensorimotor striatum (Lawrence S Kegeles et al., 2010). It is therefore proposed that excessive dopamine release in the striatum may underlie schizophrenia.

In contrast with the striatum, there is some evidence that dopamine release may be blunted in the DLPFC of patients with schizophrenia (Davis et al., 1991; Slifstein et al., 2015). As prefrontal dopamine is related to cognitive performance, it has been suggested that hypodopaminergia may contribute to cognitive deficits in schizophrenia (Goldman-Rakic and Selemon, 1997). A reformulation of the dopamine hypothesis suggests that reduced prefrontal dopamine levels may be causally linked with striatal hyperdopaminergia, (Weinberger, 1987) as cortical dopamine inhibits striatal dopamine release (Deutch et al., 1990; Karreman and Moghaddam, 1996; Kolachana et al., 1995; Wilkinson, 1997). This can also be incorporated into a stress diathesis model of schizophrenia, as deficient prefrontal dopamine levels are no longer protective against stress-induced dopamine release in the striatum, which is reported in both clinical high risk and antipsychotic-naive SZ patients but not in healthy controls (Mizrahi et al., 2012).

The phenomenological consequences of excessive striatal dopamine release and their relevance to symptoms in schizophrenia have been discussed in terms of aberrant salience (Kapur, 2003). Kapur's hypothesis builds upon extensive research implicating dopamine in mediating reward prediction, suggesting a role of dopamine in attributing salience to rewarding stimuli (Berridge 2007). In this way, excessive dopaminergic activity would result in an individual assigning meaning to innocuous stimuli leading to hallucinations, and these aberrant stimuli then being cognitively rationalised through the development of delusions (Kapur, 2003). This is further supported by a meta-analysis report of reduced fMRI activation in the ventral striatum during reward anticipation, which indicates a reduced response to rewarding stimuli in contrast to neutral stimuli of patients (Juckel et al., 2012)

1.1.2. Limitations of the dopamine hypothesis.

Firstly, although D2 receptor antagonists are efficacious in the treatment of positive symptoms in schizophrenia, with equivalent effect sizes to common medical drugs (Leucht et al., 2012), up to one third of patients do not respond to D2 receptor antagonists (Lehman et al., 2004). Medication resistance may result from accelerated drug metabolism (McCutcheon et al., 2015), but in medication resistant patients high levels of D2 receptor blockade can still be associated with no therapeutic effect (Pilowsky et al., 1993). Furthermore, clozapine is the most efficacious treatment for schizophrenia but it has less affinity for D2 receptors than many conventional antipsychotics (Pilowsky et al., 1992). These findings suggest that non-dopaminergic targets may be involved in the pathophysiology of schizophrenia.

About 50% of patients with poor responses to typical and atypical antipsychotics show an improvement when switched to treatment with clozapine, despite what is often a reduction in D2R occupancy (Pilowsky et al., 1992). The unique efficacy of clozapine may be attributed to agonism of the glycine modulatory site (Javitt, 2004). In recovered clozapine-treated patients, the addition of D-cycloserine, a partial glycine site agonist, exacerbates negative symptoms (Goff et al., 1999, 1996), which does not occur with the use of full agonists such as glycine and D-serine (Evins et al., 2000; Tsai et al., 1999). A recent study found that non-responders to medication do not possess elevated dopamine levels in the striatum (Demjaha et al., 2012), but have elevated glutamate in the anterior cingulate cortex (ACC) (Demjaha et al., 2014; Mouchlianitis et al., 2015).

Secondly, D2 blockade improves positive symptoms but has little effect on negative symptoms, impaired cognition or loss of vocational and social function (Javitt, 1999; Kim et

al., 2013; Murphy et al., 2006). This is despite patients often finding these the most debilitating aspects of disease, with their severity predicting social and functional outcome (Ventura et al., 2009). Reduced dopamine in the prefrontal cortex has been proposed to underlie cognitive symptoms in schizophrenia (Davis et al., 1991), however there is a lack of direct evidence for cortical dopaminergic alterations in schizophrenia (Kambeitz et al., 2014). Striatal hyperdopaminergia may underlie impairments in incentive motivation and cognition as indicated by animal models (Simpson et al., 2010; Ward et al., 2012), however amphetamine is found to improve negative and cognitive symptoms in patients (Barch and Carter, 2005; Laruelle et al., 1999). Thirdly, the dopamine hypothesis fails to explain neuroimaging hallmarks of schizophrenia, namely grey matter loss in cortical and medial temporal brain regions (Steen et al., 2006) and ventricular enlargement (Johnstone et al., 1976).

1.2 Glutamate

1.2.1 The NMDAR hypofunction hypothesis of schizophrenia

The N-Methyl-D-Aspartate receptor (NMDAR) hypofunction hypothesis was proposed on the basis of the psychotomimetic effects of PCP and ketamine, which are NMDAR antagonists (Anis et al., 1983). Administration of these drugs in healthy volunteers can induce positive and negative symptoms as well as cognitive impairments that are similar to those seen in patients with schizophrenia (Javitt and Zukin, 1991). When given to patients with schizophrenia, they can exacerbate these symptoms (Javitt and Zukin, 1991). The effects of PCP and ketamine thus bear a closer resemblance to the clinical features of schizophrenia than those of amphetamines (Krystal et al., 2005). These observations led to the suggestion that schizophrenia may involve hypofunction at NMDARs (Olney and Farber, 1995).

It is proposed that this NMDAR hypofunction primarily occurs on parvalbumin-expressing GABA-ergic interneurons (chandelier and basket cells) (Lisman et al., 2008), found in the cortex, hippocampus, thalamus and cerebellum (Celio, 1990; Conti et al., 1997). These cells play a pivotal role in the control of glutamatergic pyramidal cell firing, as they synapse at the perisomatic region where action potentials are initiated (Benes and Berretta, 2001). Loss of GABAergic control of pyramidal cells results in excessive glutamate release, which can act through postsynaptic AMPA glutamate receptors to trigger excitotoxic damage, see Figure 1 (Lisman et al., 2008). Excitotoxic cell loss provides a plausible mechanism for the robust cortical and hippocampal volume reductions documented in patients (Steen et al., 2006; Tregellas, 2014), although there is no direct evidence from human studies that excitotoxicity is responsible.

NMDAR function mediates hippocampal long term potentiation and long term depression required for learning and memory (Malenka and Bear, 2004), and could thus contribute to the development of cognitive impairments in schizophrenia. Parvalbumin-expressing GABA-ergic interneurons mediate theta and gamma oscillations in the brain (Buzsáki and Draguhn, 2004; Wulff et al., 2009), which bind inputs from multiple brain regions to facilitate cognitive functioning (Buzsáki and Wang, 2012) and perceptual organisation (Singer, 1999), which are disrupted in schizophrenia (Uhlhaas and Silverstein, 2005).

The NMDAR receptor is formed of four subunits, with at least one NR1 subunit, and a combination of NR2 subunits (NR2A-D) and NR3 subunits (NR3A and NR3B); with each

conformation possessing different electrophysiological properties (Dingledine et al., 1999; Matsuda et al., 2003). The NR1 subunit of the NMDAR is highly expressed throughout the brain (Moriyoshi et al., 1991), whereas NR2 subunits differ in their regional and developmental expression (Monyer et al., 1994). NR2C and NR2D subunits, preferentially expressed by hippocampal GABA-ergic interneurons, are more sensitive to glutamate and antagonist binding than their NR2A and NR2B counterparts (Dingledine et al., 1999; Grunze et al., 1996). Genetic and environmental risk factors for schizophrenia, such as hypoxic insults at birth, could modify NMDAR expression during development via excitotoxic or apoptotic neurodegeneration (Ikonomidou et al., 1999, 1989; Olney et al., 1999).

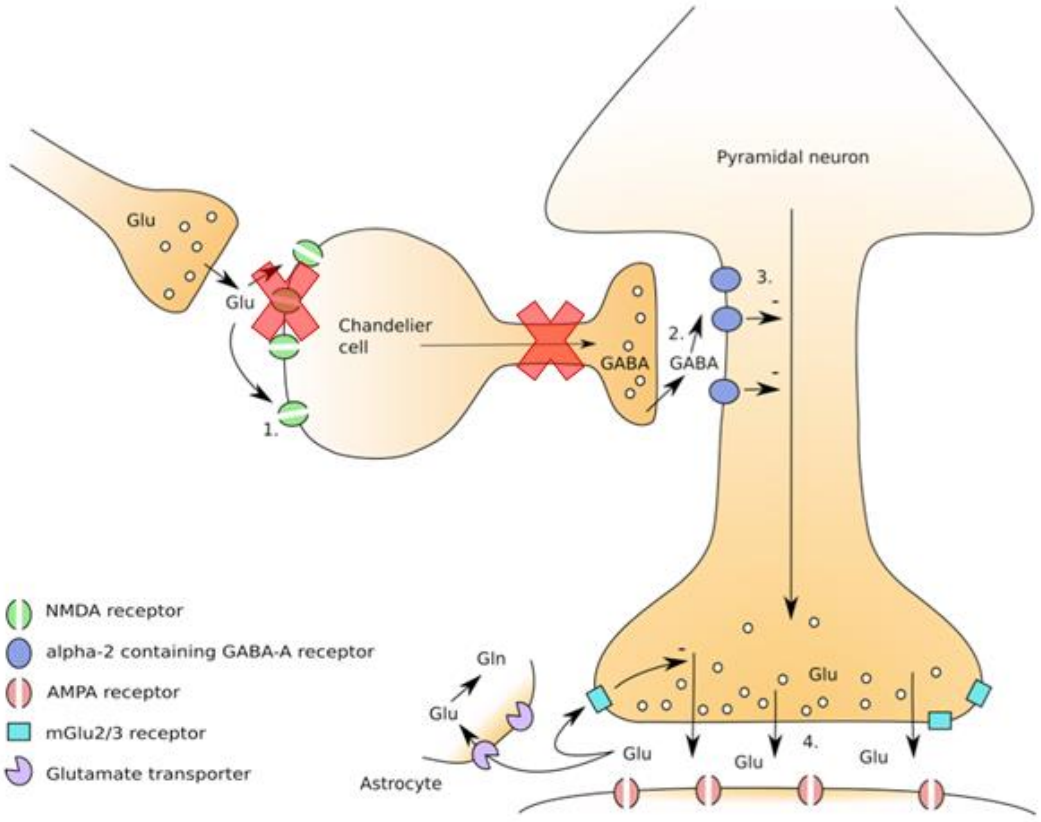

Figure 1 Glutamate hypothesis of schizophrenia, adapted from McGuire et al., 2008.
A reduction in functional NMDA receptors on Parvalbumin-expressing GABA-ergic interneurons (chandelier or basket cells) results in disinhibition of glutamatergic pyramidal cells, causing excessive glutamate release.

1.2.2 Indirect evidence for the NMDA hypofunction model of schizophrenia

Multiple lines of evidence support the involvement of glutamate dysfunction in the pathophysiology of schizophrenia.

Genetic risk loci

A recent study combined data from all previous genome-wide association studies, and identified a number of schizophrenia-associated genetic loci that converge on targets necessary for glutamatergic neurotransmission, specifically: *GRM3*; mGluR3 metabotrophic glutamate receptor, *GRIN2A*; NR2A subunit of the NMDAR, *GRIA1*; GluR1 subunit of the AMPAR, *CLCN3*; CLC-3 chloride channel located next to glutamate receptors in the hippocampus, *SLC38A7*; SNAT7 glutamine transporter involved in the cycling of neurotransmitter glutamate, *SRR*; Serine racemase catalyses D-serine, a co-agonist on NMDAR, and *CACNA1I* subunit of a T-type calcium channel, which induces LTP when co-activated with NR2B containing NMDAR (Ripke et al., 2014). As sample sizes increase, polygenic risk scores may provide future insights into the common genetic basis of schizophrenia (Dudbridge, 2013).

Post mortem findings

GABA

Post-mortem studies report reductions in presynaptic GABA-ergic interneuron markers in schizophrenia, consistent with the notion of impaired GABAergic control of glutamatergic pyramidal neurons (Coyle, 2006). Glutamic acid decarboxylase 67 (GAD67; an enzyme that synthesises GABA) in parvalbumin-expressing cells is reduced in both the prefrontal and cingulate cortex (Akbarian et al., 1995; Hashimoto et al., 2008, 2003) and the hippocampus of patients (Heckers et al., 2002). Moreover, reductions in GAD67 are localised to parvalbumin cells that co-express the NR2A subunit (Woo et al., 2004). Reduced expression of the GABA membrane transporter GAT-1 in the prefrontal cortex (Woo et al., 1998) and reduced expression of parvalbumin in the hippocampus are also found (Zhang and Reynolds, 2002) (for reviews see Benes & Berretta, 2001; Lewis, Volk, & Hashimoto, 2004; Marín, 2012). This reduction in presynaptic GABA synthesis may lead to an upregulation of GABA(A) receptors on the axon initial segments of pyramidal cells (Volk et al., 2002). The link between a loss of GABA-ergic interneuron function and NMDAR hypofunction is supported by data from animal models: chronic treatment with NMDAR antagonists downregulates GAD and GAT in the frontal cortex (Paulson et al., 2003) and reduces parvalbumin cell density (Cochran et al., 2003; Keilhoff et al., 2004).

NMDA receptors

Post mortem studies suggest that in the frontal cortex, NMDAR subunit mRNA and protein expression vary according to the specific location and subunit investigated (Kristiansen et al., 2007). Both increased (Dracheva et al., 2001) and reduced (Sokolov, 1998) NR1 subunit mRNA expression have been reported in frontal regions, with one study finding no difference in NMDAR subunit mRNA levels, although patients possessed a higher proportion of NR2D mRNA in the prefrontal cortex (Akbarian et al., 1996). NMDA receptors containing the NR2D subunit are more sensitive to ligand binding than conformations containing the NR2A and NR2B subunits (Monyer et al., 1994), which could make patients more vulnerable to excitotoxic damage in the prefrontal cortex. In regards to subunit protein expression, NR1, N2A and N2B subunits are reduced in the prefrontal cortex of patients (Errico et al., 2013), although in the orbitofrontal cortex there was no change in NR1 subunit protein expression (Toro and Deakin, 2005).

Changes in the proportion but not number of subunits may explain why a large number of studies find no difference in NMDAR ligand binding in frontal cortex (Kornhuber et al., 1989; Noga et al., 2001; Scarr et al., 2005; Simpson et al., 1991; Weissman et al., 1991). Although increased [3H]TCP and [3H]MK801 binding to the ion channel site of the NMDAR were found in orbitofrontal cortex (Simpson et al., 1991) and anterior cingulate (Zavitsanou et al., 2002), and increased [3H]glycine binding has been found in multiple frontal cortical sites (Ishimaru et al., 1994).

In the hippocampus, numerous post-mortem studies have found reduced NR1 subunit mRNA expression (Gao et al., 2000; Humphries et al., 1996; Law and Deakin, 2001) but no change in NR1 protein expression (McCullumsmith et al., 2007; Toro and Deakin, 2005). Increased NR2B mRNA expression (Gao et al., 2000) and [3H]ifenprodil binding to NR1/NR2B subunits (Grimwood et al., 1999) has been reported in patients with schizophrenia, which contrasts with one study finding no difference in 2A, 2B, 2C and 2D subunit expression (McCullumsmith et al., 2007). No differences in NMDAR ligand binding in the hippocampus have been found (Gao et al., 2000; Kerwin et al., 1990; Kornhuber et al., 1989; McCullumsmith et al., 2007; Simpson et al., 1991). This contrasts with an in vivo SPET study, which found reduced NMDAR binding in medication-free patients (Pilowsky et al., 2006), (see section 1.4.1. below for a more detailed discussion of SPET and PET findings).

In the striatum, no differences in NMDAR subunit expression (Errico et al., 2013; Meador-Woodruff et al., 2001), and NMDAR ligand binding (Meador-Woodruff et al., 2001; Noga et al., 1997; Weissman et al., 1991) have been found in patients with schizophrenia, however glycine and MK-801 binding is increased in the putamen, but not caudate or accumbens of patients (Aparicio-Legarza et al., 1998; Kornhuber et al., 1989). In the substantia nigra increased NR1 subunit mRNA expression was found in patients (Mueller et al., 2004).

NMDAR subunit alterations are present in the thalamus, and may vary according to age with increased expression in young patients but reduced in older patients (Clinton et al., 2006; Clinton and Meador-Woodruff, 2004a; Ibrahim et al., 2000). Changes in glycine/D-serine and polyamine site ligand binding, but no differences in ion channel ligand binding are found (Ibrahim et al., 2000), which together indicate a change in NMDA receptor stoichiometry but not overall expression in the thalamus.

In the right cerebellum, expression of the NR2D subunit was increased in schizophrenic patients (Schmitt et al., 2010).

In summary, ligand binding studies suggest that NMDAR expression is not grossly altered in schizophrenia, however there are significant limitations due to the multiple ligand binding sites on the NMDAR, some of which are not accessible when the receptor is inactive. The reduction of NR1 in frontal and hippocampal brain regions infers a loss of functional NMDAR in schizophrenia, as the NR1 subunit is a necessary component of the NMDAR complex (Meador-Woodruff and Healy, 2000). Post-mortem investigation of the NMDAR is further complicated by alternative splicing of the NR1 gene, which gives rise to eight different NR1 isoforms (Goebel and Poosch, 1999), the altered expression of which are beginning to be found in schizophrenia, in the ACC (Kristiansen et al., 2006) and superior temporal gyrus (Le Corre et al., 2000).

AMPA and kainate

In the hippocampus, reduced AMPAR subunit mRNA and protein expression (Breese et al., 1995; Eastwood et al., 1997a, 1997b, 1995; Harrison et al., 1991) and reduced AMPAR ligand binding (Gao et al., 2000; Kerwin et al., 1990) are found in patients with schizophrenia. Similarly reduced expression of kainate receptor subunit mRNA (Porter et al., 1997) and kainate ligand binding are found in the hippocampus (Kerwin et al., 1990; Porter et al., 1997) although one study found no difference in kainate ligand binding (Gao et al., 2000).

The consistent finding of reduced AMPAR signalling in the hippocampus may reflect a compensatory downregulation in response to excessive postsynaptic stimulation; and the loss of kainate receptors, which act presynaptically to reduce glutamate release, would make patients more susceptible to excessive stimulation. Likewise, in the thalamus, AMPA and kainate receptor subunit mRNA expression levels are reduced in patients with schizophrenia (Ibrahim et al., 2000).

In the striatum, AMPAR (Freed et al., 1993; Healy et al., 1998; Meador-Woodruff et al., 2001) and kainate receptors appear to be unchanged (Meador-Woodruff et al., 2001; Nishikawa et al., 1983; Noga et al., 1997).

In frontal cortical areas, AMPAR findings are inconsistent; no differences in AMPAR binding or subunit mRNA levels are found In the prefrontal cortex (Freed et al., 1993; Healy et al., 1998; O'Connor et al., 2007; Scarr et al., 2005), or cingulate cortex (Breese et al., 1995), which contrasts with reports of increased AMPA ligand binding in the anterior cingulate (Zavitsanou et al., 2002) and DLPFC (Noga et al., 2001). Reduced mRNA expression of GluR1 in the left superior frontal gyrus has also been reported (Sokolov, 1998).

Results are also inconsistent for kainate receptors in frontal brain regions; kainate receptor subunit mRNA is reduced in the left superior frontal gyrus (Sokolov, 1998) but no differences in mRNA subunit expression (Breese et al., 1995) or kainate ligand binding (Zavitsanou et al., 2002) are seen in the cingulate, or the DLPFC (Noga et al., 2001). However, increased kainate ligand binding is found in the frontal cortex (Deakin et al., 1989; Scarr et al., 2005).

Metabotropic glutamate receptors

A loss of mGluR2/3 receptors may predispose individuals to psychosis, as they act presynaptically to reduce glutamate release (Hackler et al., 2010). In the DLPFC however, no differences in mGluR2/3 (Frank et al., 2011) and mGluR5 ligand binding or protein levels were found in patients with schizophrenia (Matosin et al., 2013). Increased mGluR5 was found in the pyramidal cell layers of orbitofrontal cortex of patients with schizophrenia (Ohnuma et al., 1998).

In conclusion, post-mortem studies find changes in NMDA receptor stoichiometry in the frontal, hippocampal and thalamic regions, in patients with schizophrenia, as well as reduced AMPA and kainate receptors in the hippocampus and thalamus and increased presynaptic metabotrophic glutamate receptors in the orbitofrontal cortex. Alterations in

NMDAR signalling and upregulated presynaptic metabotrophic glutamate receptors could increase the vulnerability to excitotoxic damage and lead to compensatory changes, such as the downregulation of AMPA and kainate receptors .

EEG markers

Patients with schizophrenia show robust deficits in mismatch negativity (MMN); an event-related potential measured using electroencephalography (EEG) (Javitt, 2015). MMN generation is reliant upon NMDAR signalling, as the application of NMDAR antagonists to the auditory cortex in monkeys (Javitt et al., 1996), and administration of ketamine to healthy volunteers (Umbricht et al., 2000), both significantly attenuate the MMN signal. In schizophrenia, deficits in auditory sensory processing may impact upon bottom-up cognitive and attentional processing (Javitt, 2009). In healthy controls, reduced MMN amplitudes predict the severity of psychotic symptoms induced by ketamine (Umbricht et al., 2002), and in high-risk subjects predicts conversion to psychosis (Bodatsch et al., 2011).

EEG studies have shown that brain oscillations are abnormal in patients with schizophrenia (see Uhlhaas & Singer, 2010 for review). NMDAR hypofunction would be expected to disrupt brain oscillations, as their generation is reliant upon GABAergic interneurons, with parvalbumin-expressing interneurons specifically implicated in the generation of theta (5-10 Hz) and gamma (35-85 Hz) rhythms (Wulff et al., 2009). Abnormal gamma oscillations in the prefrontal cortex during working-memory task performance (Barr et al., 2010; Haenschel et al., 2009) as well as disruption of long-range gamma oscillation coordination at rest are found in patients, implicating a disconnection of frontal brain gamma with global gamma activity (Kikuchi et al., 2011). Disturbances in theta oscillations are also observed, which are responsible for establishing synchronisation across more distal brain regions (Schmiedt et al., 2005). Gamma oscillations mediate perceptual binding, necessary for face processing, selective attention, working memory, and social cognition, all of which are impaired in patients with schizophrenia (Silverstein and Keane, 2011).

Grey matter volume loss

Putative NMDAR hypofunction may lead to excessive glutamate release in the cortex, thalamus and hippocampus (Kim et al., 2011; Lisman et al., 2008; Moghaddam et al., 1997), the excitotoxic effects of which could underlie grey matter volume reductions in schizophrenia, as well as hippocampal functional hyperactivity (Konick and Friedman, 2001; Steen et al., 2006; Tregellas, 2014). Furthermore, abnormalities in hippocampal structure and function in patients relate to impairments in cognitive function (Schobel et al., 2009a)

and symptom severity (Friston et al., 1992; Schobel et al., 2009b). Chronic ketamine-challenge mice display analogous hippocampal hyperactivity and subsequent atrophy to those seen in patients. Excessive glutamate release may mediate these changes, as they are prevented by administration of LY379268; a presynaptic inhibitor of glutamate release (Schobel et al., 2009a).

1.2.3 Direct evidence for the NMDA hypofunction model of schizophrenia

NMDAR autoantibodies

More recently an autoimmune disorder associated with autoantibodies to the NMDAR has been associated with psychotic symptoms (Dalmau et al., 2008), and NMDAR autoantibodies are evident in a small proportion of patients with schizophrenia (Beck et al., 2014; Hammer et al., 2014; Steiner et al., 2013; Zandi et al., 2010).

Pharmacological models

Pharmacological models of schizophrenia, administering PCP and ketamine to healthy volunteers to cause psychotic symptoms, were fundamental to the original development of the NMDAR hypofunction hypothesis, as described in section 1.2.1 (Javitt and Zukin, 1991). The combination of pharmacological models with neuroimaging techniques has facilitated a better understanding of the mechanisms which may underlie schizophrenia. These will be outlined in the next section.

1.2.4 The effects of NMDAR-antagonists on brain function

NMDAR-antagonist administration in animals

Administration of NMDAR-antagonists in animals can inform us of the brain circuitry and neurotransmitter systems implicated in the action of these psychotomimetic drugs (for review, see Jentsch and Roth, 1999).

NMDAR antagonists increase brain metabolism, particularly in the medial frontal, thalamic and hippocampal brain regions, and to a lesser extent in the basal ganglia (Gozzi et al., 2008). Microdialysis studies (Lorrain et al., 2003; Moghaddam et al., 1997) and 13C/1H-MRS studies in rats (Chowdhury et al., 2012; Iltis et al., 2009) show that PCP induces glutamate release (as well as dopamine and serotonin) in the prefrontal cortex. When NMDAR antagonist application is restricted to the mPFC, glutamate release does not occur (Lorrain et al., 2003). It is postulated that cortical glutamate release is mediated by the thalamus (Kargieman et al., 2008) or hippocampus (Jodo, 2013; Jodo et al., 2005), via

disinhibited pyramidal projections. Furthermore, NMDAR antagonism localised to the anterior thalamus, but not the limbic cortex, causes damage to pyramidal neurons in the limbic cortex (Olney et al., 1989; Sharp et al., 2001).

The above results examine the *acute* effects of NMDAR antagonists. When chronically administered, NMDAR antagonists induce hypofunction in the medial frontal cortex and thalamus, and reduce medial frontal glutamate levels (Bustillo et al., 2012; Cochran et al., 2003; Pratt et al., 2008; Wesseling et al., 2013; Zuo et al., 2006) which may result from dendritic spine loss (Hajszan et al., 2006) or compensatory alterations in GABA function (Amitai et al., 2012). However, two other studies found chronic administration to increase medial frontal glutamate levels (Amitai et al., 2012; Chatterjee et al., 2012).

NMDAR-antagonist administration in humans

Acute administration of ketamine in healthy volunteers increases blood flow and glucose metabolism in the thalamus and in frontal regions, particularly the anterior cingulate (Holcomb et al., 2005, 2001, Långsjö et al., 2005, 2004, 2003; Rowland et al., 2010). This is replicated by fMRI reports of increased BOLD response (De Simoni et al., 2013; Malhotra et al., 1997). 1H-MRS studies find increases in both glutamate (Stone et al., 2012) and glutamine (Rowland et al., 2005) in the anterior cingulate, although one study found no differences (Taylor et al., 2012). Prefrontal cortical dysconnectivity in healthy volunteers following acute ketamine treatment better reflects the dysconnectivity observed in early rather than chronic schizophrenia patients (Anticevic et al., 2015).

Few fMRI studies have been conducted in chronic users, with two preliminary reports of reduced frontal perfusion in PCP users (Hertzmann et al., 1990; Wu et al., 1991). Chronic ketamine users have reduced grey matter and white matter integrity in the frontal cortex (Edward Roberts et al., 2013; Liao et al., 2011, 2010). However, an 1H-MRS study found no differences in ACC glutamate levels relative to controls (Stone et al., 2014).

In summary, acute NMDAR antagonism causes glutamate release in the anterior cingulate and induces activity in the medial frontal cortex and the thalamus. This finding is consistent in both humans and animals. The effects of chronic NMDAR antagonism differ from those seen with acute administration; functional studies suggest that frontal hypometabolism may occur but measures of frontal glutamate release are less consistent. Thus, if acute and chronic NMDAR antagonism have differential effects on brain circuitry, glutamate function in schizophrenia may depend on the clinical stage of disease.

1.3. Interactions between glutamate and dopamine in schizophrenia

1.3.1. The effects of limbic glutamate on striatal dopamine

It has been suggested that striatal hyperdopaminergia in schizophrenia is secondary to NMDAR dysfunction (Grace, 1991). PET studies suggest that phasic, rather than tonic dopamine levels are increased in schizophrenia, as radioligand displacement occurs at intrasynaptic rather than extrasynaptic receptors in the striatum (Abi-Dargham et al., 2000). The original model outlined by Grace proposed that reduced tonic dopamine levels in schizophrenia were compensated by an increased sensitivity of the phasic dopamine response (Grace, 1991). However, additional work revealed that concurrent increases in the tonic and phasic dopamine response cause an additive effect on phasic dopamine release (Lodge and Grace, 2006).

The hippocampus, via outputs from the ventral subiculum, regulates context-dependent responses in the striatum, by adjusting the number of spontaneously firing DA neurons in the VTA (Grace, 1991). In the MAM model of schizophrenia, disinhibited glutamatergic projections from the hippocampus cause excessive glutamate release in the ventral striatum. This leads to increased tonic dopamine release in the ventral striatum via a multisynaptic pathway, involving the disinhibition of VTA dopaminergic neurons by the ventral pallidum, see Figure 2 (Floresco et al., 2001). The number of spontaneously active dopaminergic neurons regulates the intensity of burst firing, which is NMDAR-dependent and mediated by glutamatergic input from the pedunculopontine tegmental nucleus (PPTg) (see Figure 2) (Chergui et al., 1993). PPTg activity can be altered by the prefrontal cortex and amygdala. Thus, NMDAR hypofunction can increase the 'gain' of the dopaminergic system via increased activity in descending hippocampal projections to the striatum (Floresco et al., 2003; Lodge and Grace, 2006). It is proposed that in patients with schizophrenia, increased dopaminergic tone allows more dopaminergic neurons to burst fire in response to stimuli (Schultz, 1998), making neutral stimuli appear more salient (Kapur, 2003).

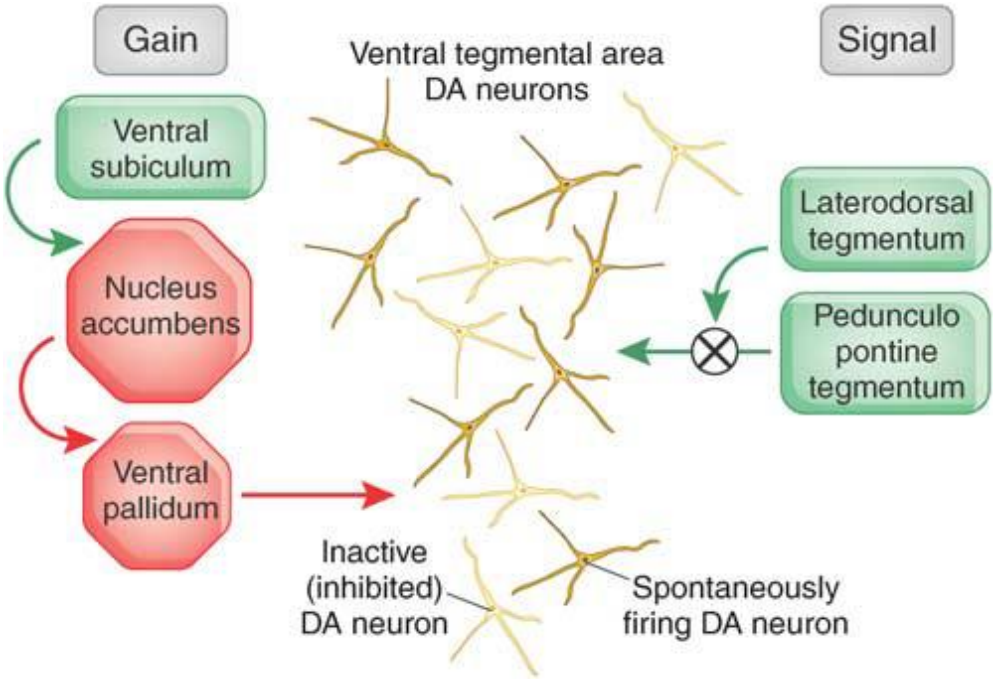

Figure 2 Adapted from Sesack and Grace, Neuropsychopharmacology, 2010 (35) 27-47.

Two pathways can alter the gain and signal of dopamine neurons in the ventral tegmental area (VTA) which project to the ventral striatum. Firstly glutamatergic drive from the ventral subiculum increases tonic dopamine levels in the ventral striatum, via disinhibition of ventral pallidum projections to allow spontaneous firing of dopamine neurons in the VTA. Secondly, the pedunculopontine tegmentum (PPTg) provides direct glutamatergic drive to increase phasic dopamine release in the ventral striatum. This requires the laterodorsal tegmentum to be active. Red represents inhibitory connections, green represents excitatory connections.

1.3.2. The effects of striatal dopamine on limbic glutamate

Excessive dopaminergic signalling in the associative striatum (L S Kegeles et al., 2010) may modulate activity in glutamatergic inputs from the cortex. Stimulation of D2 receptors downregulates glutamatergic input from the DLPFC onto striatal medium spiny neurons, reducing the influence of the DLPFC on the striatum, see Figure 3 (Laruelle et al., 2003; O'Donnell and Grace, 1994). Dysfunctional NMDAR signalling on medium spiny neurons may also contribute to this psychopathology, which has implications for cognitive functioning (Goldman-Rakic and Selemon, 1997; Weinberger and Berman, 1996).

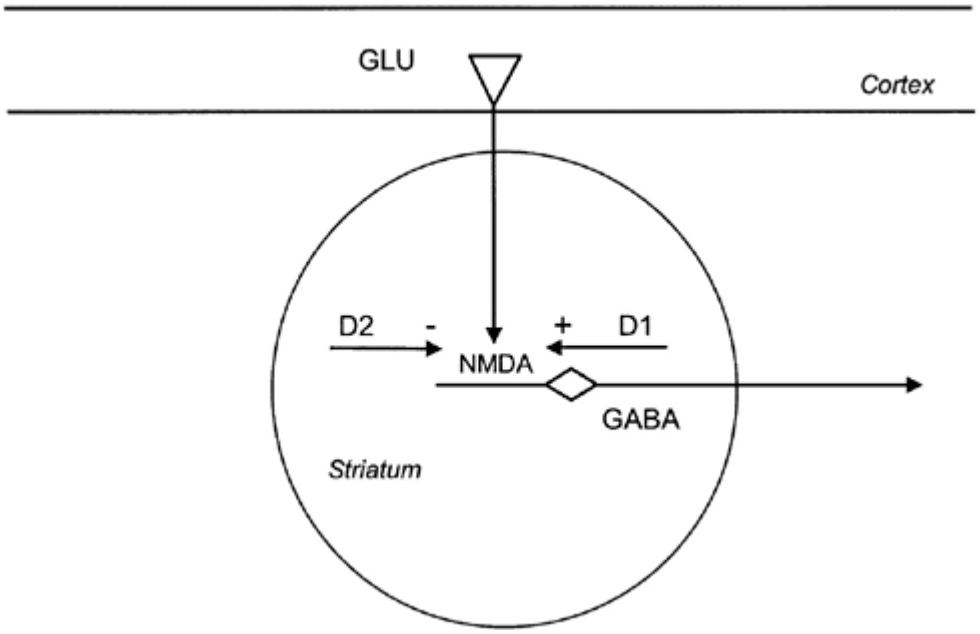

Figure 3 Adapted from Laruelle (2003), Annals of the New York Academy of Sciences 1003; 138-158.
Cortical input to the striatum is differentially modulated by D1 and D2 receptor stimulation. Excess D2 stimulation in schizophrenia reduces glutamatergic input to the striatum.

Excessive dopaminergic signalling in the striatum would also be expected to affect thalamic functioning, as this is the major output target of the striatum via the globus pallidum (Sesack and Grace, 2010). Altered glutamatergic signalling of thalamic efferents and/or NMDAR hypofunction of cortical target neurons, may underlie the reduced structural and functional connectivity of thalamo-prefrontal circuits in schizophrenia (Pergola et al., 2015; Wagner et al., 2015, 2013; Zhang et al., 2014). The thalamus is well placed to mediate many aspects of schizophrenia psychopathology, most notably sensorimotor and cognitive deficits (Allen et al., 2009).

1.3.3. Acute effects of NMDAR antagonism on striatal dopamine release

PET studies have been able to test the effects of NMDAR hypofunction on dopamine release, as postulated above. Three PET studies found that the NMDAR antagonist ketamine increases dopamine release in the striatum in healthy volunteers (Breier et al., 1998; Smith et al., 1998; Vollenweider et al.), but another three studies found no effect (Aalto et al., 2002; Kegeles et al., 2002; L S Kegeles et al., 2000). The latter result is consistent with preclinical findings, with only a small increase detected using microdialysis techniques in rats (Miller and Abercrombie, 1996), and awake rhesus monkeys (Adams et al., 2002). If dopamine levels are increased following NMDAR antagonism, the changes appear to be much smaller in magnitude than those induced by amphetamine (Adams and Moghaddam, 1998; Hertel et al., 1995). NMDAR antagonists do not directly stimulate the VTA, as NMDAR antagonists localised to this region do not induce striatal dopamine release (Freeman and Bunney, 1984; French, 1986; Zhang et al., 1992).

However, when ketamine pre-treatment is combined with an amphetamine challenge, the dopamine release induced by amphetamine is doubled (L S Kegeles et al., 2000; Miller and Abercrombie, 1996). The same results are also found when presynaptic glutamate release is blocked by mGlu2/3 agonists (van Berckel et al., 2006). This is consistent with evidence that dopamine release following amphetamine challenge in patients with schizophrenia is greater than in controls (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996; Laruelle and Abi-Dargham, 1999). Exaggerated dopamine release may occur because GABAergic inhibition of striatal dopamine release is reliant on glutamatergic signalling between the PFC and VTA. This lead to the proposal that abnormalities in prefrontal control systems may lead to striatal hyperdopaminergia in schizophrenia (Carlsson et al., 1999).

1.4. Neuroimaging studies of the glutamate system

1.4.1. Positron Emission Tomography

Development of PET tracers to investigate NMDAR is challenging, as current radiotracers show high non-specific binding and poor signal to noise ratios (Bressan and Pilowsky, 2000; Waterhouse, 2003). To date, there has been one study using the SPET [123I]CNS-1261 radiotracer in schizophrenia; where total NMDAR binding in clozapine treated patients (Bressan et al., 2005) and NMDAR binding in the hippocampus of medication-free patients were reduced relative to controls (Pilowsky et al., 2006). Reduced total NMDAR binding approached significance in patients treated with typical antipsychotics, and negatively correlated with total and negative PANSS score (Bressan et al., 2005; Pilowsky et al., 2006). However these NMDAR PET ligands have been difficult to validate, as the channels must be open for ligand binding to occur. Further NMDAR PET tracers are currently in development (McGinnity et al., 2015, 2014), as well as probes for metabotrophic glutamate receptors, which may serve to validate these studies in the future (Fuchigami et al., 2015; Li et al., 2012). An alternative strategy to measure brain glutamate levels is to use Proton Magnetic Resonance Spectroscopy (1H-MRS).

1.4.2. Proton Magnetic Resonance Spectroscopy (1H-MRS)

1H-MRS is a non-invasive technique that is able to measure brain metabolites above 0.5mmol, including; N-acetyl-aspartate (NAA); implicated in neuronal integrity and function, creatine (Cr); involved in energy homeostasis, myo-inositol; forms intracellular second messengers and acts as a glial marker, choline; indicates membrane turnover, and lactate; which signals anaerobic glycolysis, see Figure 4. 1H-MRS is also able to measure certain neurotransmitters; glutamate, the major excitatory neurotransmitter, and its metabolite glutamine, and their combination Glx, and most recently GABA, the major inhibitory neurotransmitter, using specialised editing sequences. In single voxel spectroscopy (SVS), metabolites are measured in a predetermined voxel of interest, whereas MRSI (magnetic resonance spectroscopic imaging) acquires metabolite data from a whole brain slice. MRS can utilise the resonant frequencies of numerous elements; phosphorus (31P) (to assess cell membrane integrity), carbon (13C) (to determine glutamate metabolism), fluorine (19F), sodium (23Na) and protons (1H), with the latter method being the most widely used due to the abundance of protons in organic tissue

metabolites (van der Graaf, 2010). Single voxel 1H-MRS measurement of glutamate and its metabolites will be the focus of this thesis.

1.4.3. 1H-MRS signal acquisition and analysis

1H-MRS is an extension of MRI imaging; when a tissue is exposed to an external magnetic field (B0), protons align along the direction of the applied field and resonate at a frequency that is given by the Larmor equation, with the Larmor frequency depending on the external magnetic field (B0) and the local microenvironment. When an additional magnetic field is applied in a perpendicular orientation (B1) in the form of a radiofrequency pulse, the protons will flip orientation. The proton will return back to the original orientation and precess back around B0, which induces current to produce a signal. This signal decreases (free induction decay) and is converted into a magnetic resonance spectrum via Fourier transform (Marsman, 2013). (See http://www.drcmr.dk/MR for animation)

The interactions of protons with the surrounding molecules cause a change in the local magnetic field, leading to a change of the proton's Larmor frequency (called chemical shift). The chemical shift gives information about the molecular group carrying the proton and is expressed in parts per million (ppm). Ppm is independent of field strength, ie at both 1.5T and 3T, glutamate peaks are present at 2.35ppm and 3.75ppm, corresponding to the coupled spins of the C2–C4 hydrogen nuclei (see

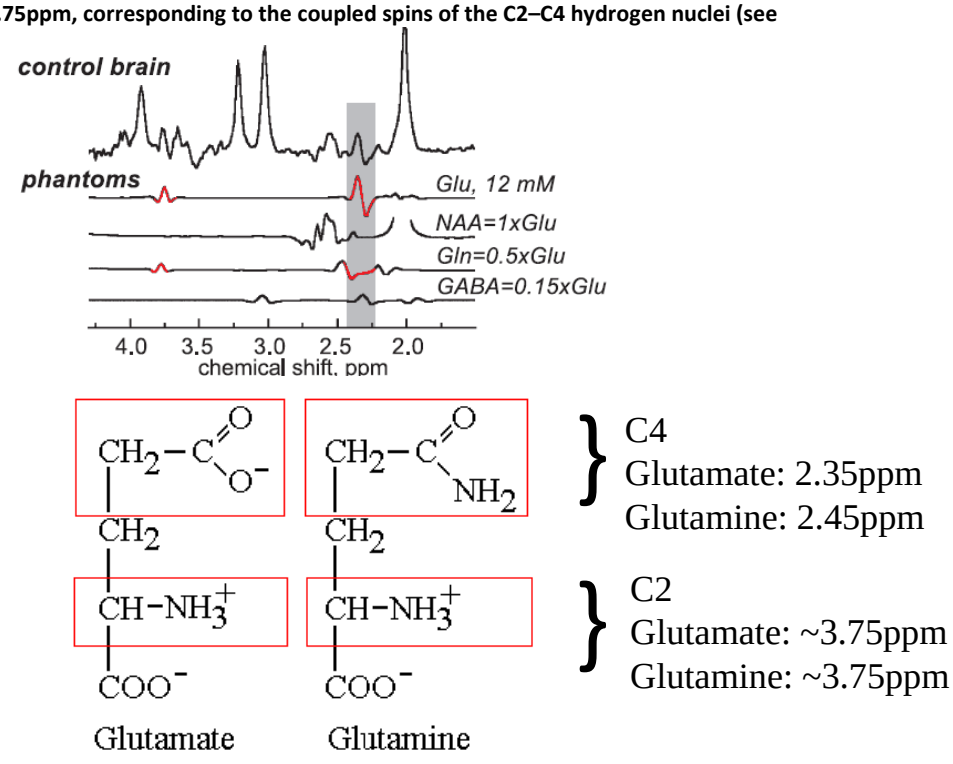

Figure 5). Thus individual metabolites have signature chemical shifts. The range of chemical shifts for physiological metabolites is narrow (2-4ppm) and so metabolite chemical shifts often overlap (van der Graaf, 2010).

Field strengths below 3T cannot resolve well the resonant frequencies of glutamate from those of glutamine, and so these metabolites are instead reported in their combination as Glx. At field strengths of 1.5 to 3 T, the glutamate signal accounts for the majority (80-90%) of the Glx signal (Snyder and Wilman, 2010a). At 3T the glutamate and glutamine peaks can be largely resolved, although the overlap still makes it difficult to accurately obtain glutamine values, as 20% of the glutamine signal is contaminated by glutamate, whereas only 10% of the glutamate signal is contaminated by glutamine (Snyder and Wilman, 2010a). At 4.7T and above, glutamine resonant frequencies are clearly separate from those of glutamate and so measures can be obtained of both (Snyder and Wilman, 2010a). 1H-MRS provides a measure of total concentrations within a relatively large region of interest (typically 2mm x 2mm x 2mm), and thus the concentration estimates reflect both intracellular and extracellular glutamate, as well as in grey matter, white matter and CSF (depending on its location). Furthermore 1H-MRS cannot differentiate between glutamate that is involved in metabolism, and that involved in neurotransmission (Rothman et al., 2011). Glutamine, however, can act as an indirect measure of neurotransmitter glutamate turnover, as 80% of glutamine is used for glutamate neurotransmitter cycling (Rothman et al., 2011).

As well as field strength, there are a number of methodological parameters to consider when using 1H-MRS. For high spectral resolution, short echo times at high field strengths increase the signal to noise, field homogeneity must be maximised by 'shimming' direct currents in the gradient and shim coils, and the large water signals that arise from high water concentrations should be supressed. The acquisition sequence, Point Resolved Spectroscopy (PRESS), has a better signal to noise ratio than Stimulated Echo Acquisition Mode (STEAM), however STEAM can achieve shorter echo times with more precise volume selection (Schwerk et al., 2014). The edited PRESS sequence MEGA-PRESS is designed to resolve GABA whereas the PRESS sequence is more suited to measuring glutamate (Henry, Lauriat et al. 2011).

The selection of analysis software may also affect the output measures, as the model used to fit the spectra will vary between programs. One study found that the output from LCModel version 6.1-4F was more reproducible when measuring Glx compared to the Amares algorithm in jMRUI software (O'Gorman, Michels et al. 2011). LCModel compares spectra to a basis set acquired using a phantom (Provencher, 2015). The area under the spectrum peak is proportional to a metabolites concentration. A metabolites value is normalised by scaling metabolites to the unsuppressed water peak and reported as

'institutional units'. If absolute concentrations are unknown, concentration ratios are reported. Creatine is commonly used as a reference as it is relatively stable in the brain. LCModel estimates the standard deviations of fitted metabolites (Cramer-Rao lower bounds: CRLB) expressed in percent of the estimated concentrations. A metabolite concentration with a CRLB of <20% is considered acceptable. Lastly, as the presence of cerebrospinal fluid (CSF) in the voxel would underestimate metabolite concentrations when absolute values are reported, it is important to correct for differences in tissue composition.

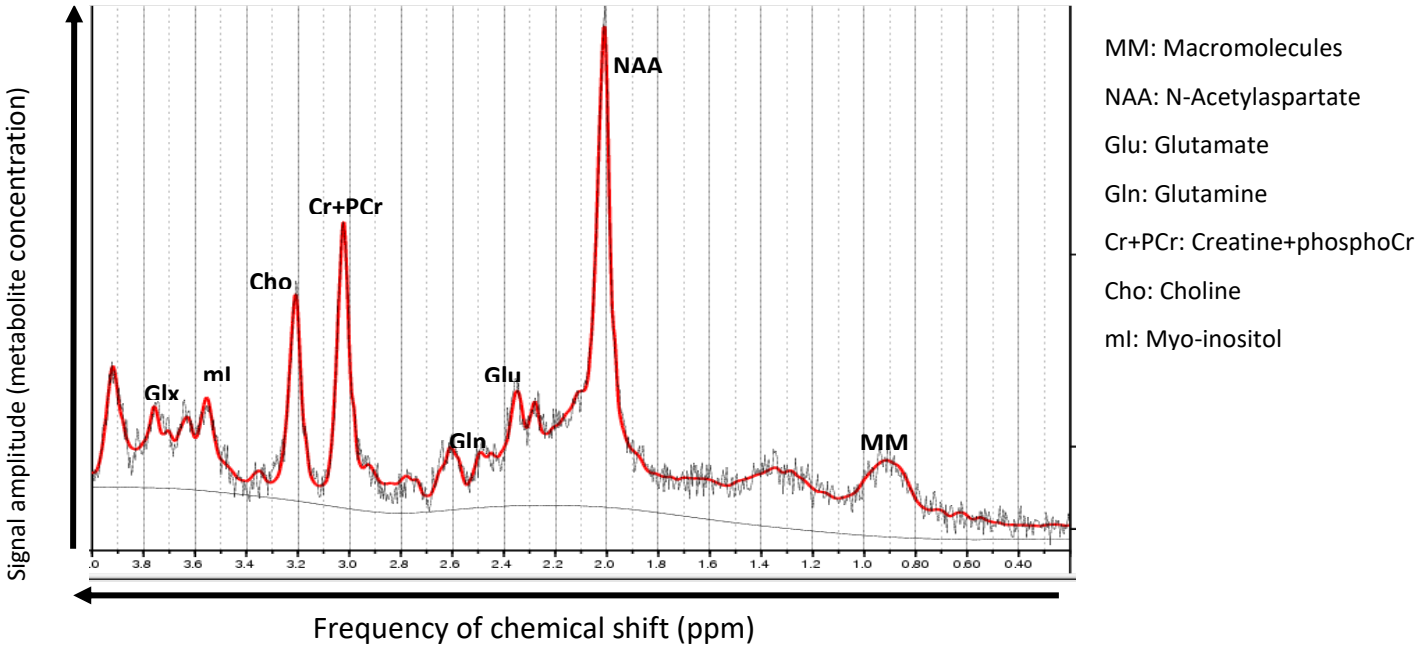

Figure 4 1H-MRS spectra acquired at 3T (PRESS) from the brain of a healthy control.
The main metabolites are labelled. LCModel (Provencher) fits the output (black) to a basis set of 16 metabolites to create a modelled spectra (red) to acquire metabolite estimates.

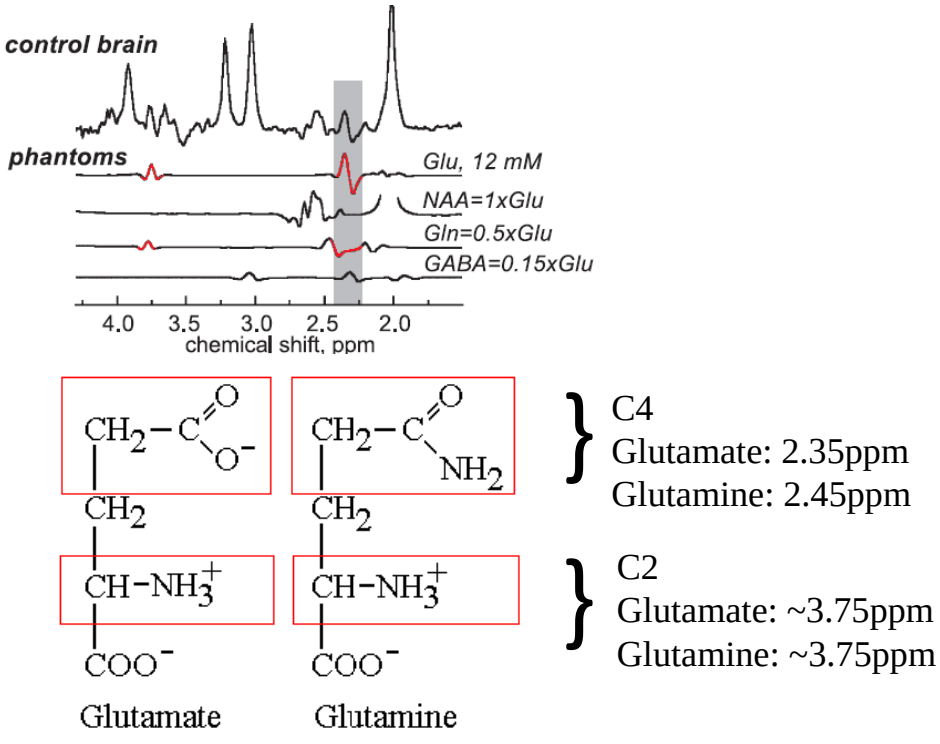

Figure 5 1H-MRS PRESS spectra, adapted from Schubert, Gallinat, Seifert, & Rinneberg, 2004.
PRESS spectra (echo time = 80ms) from a healthy volunteers' brain is shown in comparison to phantom acquisitions of individual metabolites, to allow visualisation of overlapping peaks. Chemical shifts for the coupled spins of the C2–C4 hydrogen nuclei in glutamate and glutamine are shown. Quantification of glutamine and glutamate targets the C4 proton multiplet resonance, as this is the region of least overlap.

1.4.4. 1H-MRS regions of interest

The anterior cingulate cortex (ACC) integrates information from the emotional limbic system (predominantly rostral ACC) and cognitive prefrontal regions (predominantly caudal ACC), see Figure 6 (Stevens et al., 2011). The rostral ACC is connected to many regions implicated in schizophrenia psychopathology, namely the hippocampus, ventral striatum and amygdala (see section 1.3 for an outline of how this circuitry may be compromised in schizophrenia). According to the NMDAR hypofunction model of schizophrenia, acute NMDAR antagonist administration to healthy controls increases both 1H-MRS measures of glutamate (Stone et al., 2012) and glutamine (Rowland et al., 2005) in the anterior cingulate cortex (see section 1.2.4). Furthermore grey matter reductions in the ACC have been reported in patients with schizophrenia (Fornito et al., 2009).

The thalamus processes and integrates sensory input with information from higher-order limbic regions via a series of modality specific thalamo-cortical loops. The thalamus is therefore well placed to underlie the disturbances in sensory experience seen in schizophrenia (Pergola et al., 2015). Thalamo-cortical projections are glutamatergic, whereas thalamic tone is maintained by GABAergic interneurons (Clinton and Meador-Woodruff, 2004b). The thalamus is strongly implicated in the NMDAR hypofunction model of schizophrenia, as NMDAR antagonist induced glutamate release in the medial frontal region is thought to be mediated by the thalamus (Kargieman et al., 2008). Furthermore post-mortem studies report reduced ionotropic glutamate receptor subunit gene expression in thalamocortical relay neurons (Sodhi et al., 2011).

Lastly, NMDAR antagonist administration to healthy volunteers increases metabolism in the anterior cingulate and thalamus (Deakin et al., 2008; De Simoni et al., 2013; Holcomb et al., 2005, 2001). The ACC and thalamus are therefore suitable regions of interest to investigate the presence of glutamatergic metabolite alterations in schizophrenia, and thus will be examined in the studies described in Chapter 4 and 5.

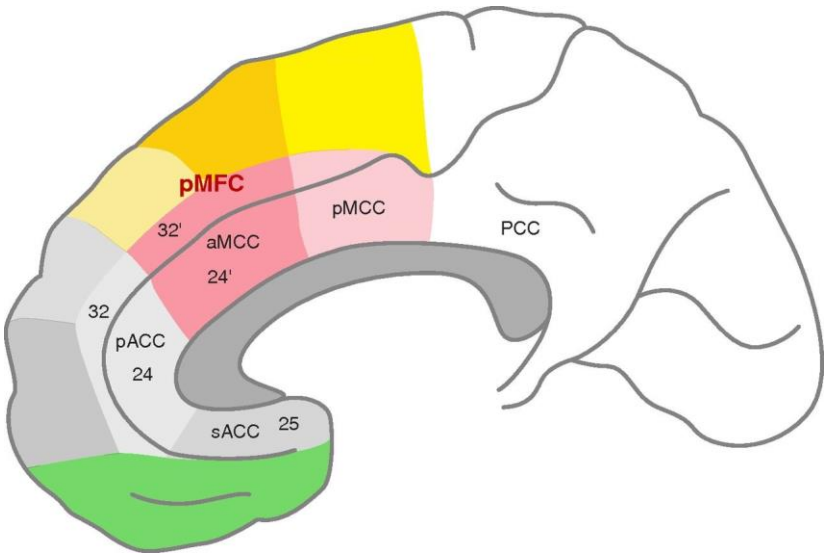

Figure 6 Adapted from Ullsperger et al., Physiological Reviews (2014) 94, 1, 35-79.

The cingulate cortex has been divided into rostral (grey) and caudal (pink) portions, the posterior cingulate cortex (PCC) is also shown. The rostral anterior cingulate cortex is subdivided into the subcallosal (sACC) and pregenual (pACC) anterior cingulate cortices. The caudal ACC is further subdivided into the anterior and posterior midcingulate cortices (aMCC and pMCC, respectively).

1.5. 1H-MRS glutamate findings in schizophrenia

The previous meta-analysis of 1H-MRS glutamate measures in schizophrenia found decreases in glutamate and increases in glutamine in the medial frontal cortex of patients compared to controls (Marsman et al., 2013). Furthermore, medial frontal glutamate and glutamine in schizophrenia decreased with age at a faster rate relative to controls (Marsman et al., 2013). These findings indicate increased glutamate turnover in schizophrenia, as glutamate is converted into glutamine (Bak et al., 2006). This is consistent with the NMDAR hypofunction model, as acute ketamine was found to increase 1H-MRS measures of glutamate (Stone et al., 2012) and glutamine (Rowland et al., 2005) in the anterior cingulate of healthy controls.

This meta-analysis examined studies published up until 2011, and therefore an update of the literature is needed. Glutamatergic differences in the basal ganglia and cerebellum could not be examined due to insufficient studies at the time, and glutamine was not examined in the thalamus and medial temporal lobe. Previous studies comparing patient groups indicate that glutamate metabolite differences may vary with the stage of the disorder (Natsubori et al., 2014; Ohrmann et al., 2007, 2005; Stanley et al., 1996; Szulc et al., 2004), however clinical groups could not be analysed separately in the meta-analysis due to insufficient study numbers. It is not currently established whether antipsychotic medication affects glutamate levels. Longitudinal MRS studies have not found effects of antipsychotic treatment on medial frontal glutamatergic measures (Bustillo et al., 2010; Theberge et al., 2007), although medication effects have been reported in the striatum (de la Fuente-Sandoval et al., 2013). One previous study reported higher medial frontal Glx in unmedicated relative to medicated patients (Kegeles et al., 2012). Lastly, numerous studies have tested the relationship between glutamate measures and symptom severity, however, a review of the literature has not yet been conducted.

Therefore, I will conduct an updated case-control meta-analysis of all published reports of regional glutamatergic measures in high risk, first-episode psychosis and schizophrenia (Chapter 2.2). In addition, I will assess the influences of age, symptom severity, and antipsychotic treatment on 1H-MRS glutamate measures using systematic review (Chapter 2.1), meta-regression (Chapter 2.2), and multicentre analyses (Chapter 2.3).

1.6. Predicting the response to antipsychotic medication

Approximately one third of patients with schizophrenia do not respond to antipsychotic treatment, despite sufficient blockade of D2 receptors (Pilowsky et al., 1993). This presents an important clinical challenge, as these patients have poorer outcomes and incur large economical costs (Caspi et al., 2004). At present treatment response cannot be predicted on the basis of clinical features. Instead, treatment response has to be determined empirically, by a lengthy process of evaluating one antipsychotic followed by at least one other. In practice, many patients are not fully adherent, the doses prescribed are not optimal, or the drug is not given long enough to work (Kane, 2012). There is thus a great need for tools that are able to predict drug response before treatment starts. This would allow non-responders to be fast tracked to receive clozapine. Clozapine is currently the only drug licensed for treatment-resistant patients, however due to its risk for serious adverse events that require close monitoring, its use is often delayed as other antipsychotics are trialled first (Howes et al., 2012b).

Neuroimaging measures of dopamine and glutamate function offer promising candidates in the prediction of treatment response. Recent findings suggest that striatal dopamine levels may not be elevated in treatment non-responders (Demjaha et al., 2012), but instead may be characterised by elevated glutamate levels in the anterior cingulate cortex (Demjaha et al., 2014; Egerton et al., 2012). However, it is not yet known whether glutamate differences between responders and non-responders predate antipsychotic treatment or were secondary to this. Of the two methods that may be used as predictive tools, 1H-MRS measurement of glutamate is less invasive and less expensive than PET, and requires a 3T MRI scanner which are widely available. PET is far superior in its molecular specificity; it can specifically measure dopamine synthesis or release, whereas MRS provides the average concentration of glutamate and glutamine in a voxel (discussed above in section 1.4.4). However, there are relatively few PET scanners, even in developed countries, with scans costing £3-8,000 per subject, compared to £500 for a 1H-MRS scan. A further disadvantage of PET is that it involves exposing the subject to radiation, limiting the number of scans per subject, and its use in females who may be pregnant.

1.7. Glutamatergic drug targets for the treatment of schizophrenia

Because of the evidence implicating glutamate dysfunction in schizophrenia, there has been great interest in evaluating the effects of drugs that act on the glutamate system in schizophrenia patients. The glycine/D-serine modulatory site on the NMDAR provides a suitable target, and compounds including glycine, D-serine, or D-cycloserine, and glycine transport inhibitors have been assessed. In addition mGluR2/3 agonists may be effective in preventing neurotoxicity as they reduce presynaptic release of glutamate (Javitt et al., 2012).

To date, effects of glutamatergic compounds have been small to modest; D-serine shows a moderate effect in reducing negative, but not positive symptoms (Tuominen et al., 2005), however recent trials of an mGluR2/3 agonist (Downing et al., 2014) and glycine reuptake inhibitor, Bitopertin, have been negative (Goff, 2014). Small to moderate effect sizes may be due to trials mainly recruiting chronic patients who are already receiving antipsychotics, and compounds acting on glutamatergic targets may be more effective in medication naïve first episode patients (Grace, 2015). Indeed, in a monotherapy trial of sarcosine (a glycine transporter-I inhibitor), 5 of 11 acutely symptomatic patients showed a 20% improvement in PANSS total scores. Of the patients who responded, all of these were antipsychotic-naïve, whereas all non-responders had previous antipsychotic exposure (Lane et al., 2008).

Recent evidence suggests that sodium nitroprusside augmentation of nitric oxide levels, a downstream product of NMDAR activation, may have therapeutic effects in schizophrenia (Hallak et al., 2013). Minocycline also has therapeutic effects in schizophrenia, and is thought to indirectly modulate NMDAR due to its ability to prevent NMDAR-antagonist induced neutotoxicity and injury in cell culture, and reduce NMDAR-antagonist cognitive deficits in animal models (Chaves et al., 2009). Further studies in larger patient samples are needed to replicate efficacious findings in patients.

1.8. Conclusions and study rationale

Predictive tools are needed in schizophrenia to identify the third of patients who will not respond to treatment. This would facilitate immediate access to clozapine which effectively treats this patient cohort, which is not usually prescribed until two other antipsychotics have been trialled. Recent studies implicate that 1H-MRS glutamate levels differ in responders and non-responders to treatment (Demjaha et al., 2014; Egerton et al., 2012; Mouchlianitis et al., 2015). However these findings are in medicated patients, and so it is unclear whether glutamate levels predict response in antipsychotic naïve patients. The differences between groups may instead reflect differences in symptomatology at presentation, or represent a longitudinal reduction in glutamate in those who respond. Therefore, longitudinal studies following antipsychotic naïve patients are needed to determine whether baseline 1H-MRS glutamatergic measures predict treatment response.

Aims and Objectives

This thesis aims

1. To critically review and conduct a meta-analysis of the glutamate proton magnetic resonance (1H-MRS) literature in schizophrenia.
2. To investigate the relationships between regional glutamate concentrations and symptom severity, in a large multicentre dataset.
3. To investigate the repeated-measures reproducibility and reliability of using proton magnetic resonance spectroscopy (1H-MRS) to measure glutamate in vivo.
4. To examine the relationship between 1H-MRS glutamate measures and the acute and medium-term response to antipsychotic medication.

Outline of this thesis

Chapter 2 presents two published papers and a multicentre analysis. The first paper reviews the relationship between published reports of glutamate and symptom severity, as well as cognitive function. The second paper provides a meta-analysis of all published reports of regional glutamatergic measures in those at high risk for schizophrenia, with first episode psychosis and in chronic schizophrenia. The final section of Chapter 2 outlines a multicentre analysis, which examines the relation of 1H-MRS glutamate measures to symptom severity and medication status in a large dataset of individual patient data collected at multiple centres.

In **Chapter 3**, the reliability and reproducibility of 1H-MRS measures of glutamate, glutamine and Glx were assessed over a long time period of several months. CSF-corrected Glx was found to be the most reliable measure, and a power analysis informed the study design presented in **Chapter 4**. Chapter 4 describes the first longitudinal study that examines 1H-MRS glutamate measures and the acute and medium-term response to antipsychotic medication. Finally in **Chapter 5**, the results of the preceding chapters are discussed.

CHAPTER 2 - Proton Magnetic Resonance Spectroscopy (1H-MRS) in Schizophrenia

This chapter comprises 2 published papers and an additional study, all of which aim to summarise the current findings of 1H-MRS studies measuring glutamate in schizophrenia. The first paper (section 2.1) reviews studies which have examined glutamate levels in schizophrenia and the relationship with symptom severity and cognitive function. The second paper (section 2.2) provides a meta-analysis of all published reports of regional glutamatergic measures in schizophrenia. The third study (section 2.3) builds from the previous sections and examines the relation of 1H-MRS glutamate measures to symptom severity and medication status in a large dataset of individual patient data collected at multiple centres.

2.1. Relationship between glutamate dysfunction and symptoms and cognitive function in psychosis

Kate Merritt

Philip McGuire

Alice Egerton

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Relationship between glutamate dysfunction and symptoms and cognitive function in psychosis

Kate Merritt*, Philip McGuire and Alice Egerton

Department of Psychosis Studies, Institute of Psychiatry, King's College London, London, UK

Edited by:
André Schmidt, University of Basel, Switzerland

Reviewed by:
John H. Krystal, Yale School of Medicine, USA
Anouk Marsman, Johns Hopkins University, USA

****Correspondence:***
Kate Merritt, Department of Psychosis Studies, Institute of Psychiatry, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, UK
e-mail: kate.merritt@kcl.ac.uk

The glutamate hypothesis of schizophrenia, proposed over two decades ago, originated following the observation that administration of drugs that block NMDA glutamate receptors, such as ketamine, could induce schizophrenia-like symptoms. Since then, this hypothesis has been extended to describe how glutamate abnormalities may disturb brain function and underpin psychotic symptoms and cognitive impairments. The glutamatergic system is now a major focus for the development of new compounds in schizophrenia. Relationships between regional brain glutamate function and symptom severity can be investigated using proton magnetic resonance spectroscopy (1H-MRS) to estimate levels of glutamatergic metabolites *in vivo*. Here we briefly review the 1H-MRS studies that have explored relationships between glutamatergic metabolites, symptoms, and cognitive function in clinical samples. While some of these studies suggest that more severe symptoms may be associated with elevated glutamatergic function in the anterior cingulate, studies in larger patient samples selected on the basis of symptom severity are required.

Keywords: schizophrenia, psychosis, glutamate, NMDA, MRS, spectroscopy, imaging

INTRODUCTION

Accumulating evidence suggests that glutamatergic dysfunction may contribute to the pathogenesis of schizophrenia, and the symptoms and cognitive deficits associated with the disorder (1). The glutamatergic system presents an attractive therapeutic target, as dopaminergic antipsychotics have little effect on negative symptoms or cognitive impairment, yet these features are better predictors of social and functional outcome than positive symptoms (2, 3). Here we briefly review the existing evidence linking abnormal glutamatergic transmission to cognitive, negative, and positive symptoms of schizophrenia.

The observation that administration of antagonists at the *N*-methyl-d-aspartate glutamate receptor complex (NMDAR) such as phencyclidine (PCP) or ketamine to healthy volunteers induces effects which resemble aspects of schizophrenia symptomatology forms a cornerstone of the glutamate hypothesis of schizophrenia (4–6). NMDAR antagonists also worsen positive, negative, and cognitive symptoms in patients with schizophrenia (7, 8). While dopamine-stimulating drugs such as amphetamine also produce positive "psychotic"-like effects, negative-type symptoms, and cognitive deficits are far more prominently elicited by ketamine than amphetamine administration (9). Neuroimaging studies indicate that these effects of ketamine are mediated by changes in activity in the frontal and cingulate cortices and the thalamus (10–13).

While pharmacological studies have provided evidence linking NMDAR dysfunction to these symptom domains, direct associations between glutamatergic function and symptom severity may be provided by neuroimaging studies. In a single photon emission tomography study using the NMDAR radiotracer ^{123}ICNS-1261 in schizophrenia, the availability of NMDAR in the hippocampus was negatively associated with the severity of symptoms, especially negative symptoms (14). A ketamine challenge study in healthy volunteers using the same radiotracer linked regional NMDAR binding to the induction of negative (but not positive) symptoms, particularly in the thalamus (15). These approaches are currently limited by a lack of availability of suitable radiotracers (1).

An alternative is to use proton magnetic resonance spectroscopy (1H-MRS) to estimate the concentration of glutamatergic metabolites. MRI scanners with field strengths of 3T or above can resolve glutamate, at least for the most part, from its metabolite glutamine (16). At lower field strengths glutamate and glutamine are reported in combination, as Glx. A limitation of 1H-MRS is that the glutamate concentration estimates are not specific to neuronal glutamate, and that changes in glutamate levels cannot be specifically attributed to altered neurotransmission over other metabolic processes (17). However the majority (~80%) of glutamine synthesis

reflects cycling of neurotransmitter glutamate (17), and clinical studies at 4T in minimally treated patients with schizophrenia have reported higher Gln/Glu ratios (18) or higher glutamine levels (19) in the anterior cingulate cortex (ACC). This is consistent with increased glutamatergic neurotransmission, but could also result from a deficiency in the conversion of glutamine to glutamate. However the majority of 1H-MRS studies in psychosis have used field strengths <4T and thereby were unable to accurately quantify glutamine concentrations. Thus, the results from these studies at lower field strengths cannot be specifically attributed to changes in glutamate neurotransmission. Nonetheless, increases in frontal glutamatergic neurotransmission in schizophrenia are broadly consistent with the NMDA receptor hypofunction hypothesis, as administration of NMDAR antagonists to rats increases glutamate release as detected by microdialysis (20), and 1H-MRS studies report elevated Gln/Glu ratios (21), or increased glutamate levels with no changes in glutamine (22). 1H-MRS studies with ketamine in man at 4T reveal increased ACC glutamine (23), and at 3T reveal increased ACC glutamate (24). One mechanism through which increases in glutamate release may occur is via NMDAR dysfunction on GABA-ergic interneurons, leading to the disinhibition of glutamatergic pyramidal cells, as described by the NMDAR hypofunction hypothesis (25).

Individual 1H-MRS glutamate studies in schizophrenia have produced some inconsistent findings. In general, studies at higher field strengths suggest that frontal glutamine function is elevated in the early stages of psychosis (18, 19), whereas the findings in chronic schizophrenia are more variable (26–30). This could reflect effects of antipsychotic medication. For example, one crosssectional and one longitudinal study reported higher Glx levels in the PFC of unmedicated, but not medicated schizophrenia, in comparison to controls (31, 32), although longitudinal studies have reported no effect of medication on anterior cingulate glutamate or glutamine levels (18, 33, 34). It could be that symptom severity contributes to variability in glutamatergic metabolites between patients early or late in the illness, as samples involving patients in the early phase of psychosis usually comprise non- or minimally medicated patients who are relatively symptomatic, whereas studies in chronic schizophrenia often involve patients who have been treated for long periods and have less severe or more stable symptoms (1). In relation to this, a recent meta-analysis of 1H-MRS glutamatergic studies in major depressive disorder found lower levels of Glx in the ACC which were only significant when remitted patients were excluded from the analysis (35).

In order to better understand the possible relationships between 1H-MRS glutamate, glutamine, and Glx levels and symptom severity, we identified publications that have reported associations with severity along symptom domains. These included studies in individuals at risk of psychosis, and patients with first-episode psychosis or established schizophrenia (**Table 1**).

RELATIONSHIPS BETWEEN GLUTAMATE FUNCTION AND POSITIVE SYMPTOMS

Several studies have investigated associations between regional glutamatergic metabolite levels and the severity of positive psychotic symptoms (**Table 1**), and most found no association between regional Glx, glutamate or glutamine levels and positive symptom severity, in either high genetic or clinical risk populations (36, 37), first-episode psychosis (18, 19, 37–40) or chronic schizophrenia (26–28, 30, 41–46).

However, many of these studies involved small patient samples, relied on *post hoc* correlational analyses, and patients in whom the severity and/or the variance in severity of symptoms was low, due to either being sub-clinical threshold in the at risk studies or due to the presence of antipsychotic medication in established schizophrenia. A recent study pooling both medicated and unmedicated patients, where unmedicated patients possessed elevated Glx in medial prefrontal cortex (mPFC), detected an association between positive symptom severity and mPFC Glx, although this did not survive correction for multiple comparisons (31). Another study in the PFC found that treatment reduced the level of Glx/Cr in chronic patients, and associated the change in Glx with improvement in total BPRS score (32). Moreover a recent study found that 4 weeks of antipsychotic treatment in first-episode psychosis patients reduced Glx in the striatum, and this was associated with improvement in PANSS score (47).

A notable exception is the study of Ota et al.(48), which directly compared patients experiencing exacerbated psychotic symptoms to healthy controls and stable patients, and found that increases in Glx in inferior parietal white matter were specific to the group currently experiencing exacerbated psychotic symptoms (48). In line with this, our own studies which have compared glutamate levels in patients according to symptom severity have found higher ACC glutamate levels in first-episode psychosis patients who are still symptomatic following treatment compared to those in remission (38) and in patients with treatment-resistant schizophrenia compared to those who respond to medication (49). These differences may be independent of medication effects, as groups either did not differ according to medication (38, 49) or the symptomatic group were actually receiving higher medication doses (48), and as longitudinal studies have not reported changes in cortical glutamate in relation to changes in positive symptoms following antipsychotic treatment (18, 33, 34). This suggests that glutamate and Glx levels may be selectively elevated in patients whose positive symptoms are not well controlled by conventional antipsychotic medication.

RELATIONSHIPS BETWEEN GLUTAMATE FUNCTION AND NEGATIVE SYMPTOMS

We recently reported that greater severity of PANSS negative symptoms was associated with higher levels of glutamate in the ACC in first-episode psychosis (38). Although there were several methodological differences, this contrasts with the study of Reid et al. (26), which reported that negative symptoms were associated with *lower* levels of ACC Glx in chronic schizophrenia. Other studies investigating correlations between ACC glutamatergic metabolites and negative symptoms have found no significant relationship (18, 19, 28, 42, 46, 50). We are not aware of any studies which have specifically compared regional levels of glutamatergic metabolites in patient groups selected according to differences in negative symptom severity.

As listed in **Table 1**, in brain regions other than the ACC many studies have failed to detect significant relationships between negative symptoms and regional glutamate, glutamine, or Glx levels in high genetic or clinical risk groups (36, 37), first-episode

Table 1 | Summary of articles reporting high levels of glutamate metabolites associated with greater or lesser severity of symptoms.

Reference	Field strength	Population	*n*	Brain region	Metabolite	Measure	Direction
Tandon et al. (54)	1.5	Familial high risk	23	Thalamus	Glx	SIPS CSS	+ NS
				Caudate	Glx	SIPS, CSS	+
				ACC	Glx	SIPS, CSS	NS
Yoo et al. (36)	1.5	GHR	22	ACC, DLPFC, thal	Glx	PANSS, BPRS	NS
de la Fuente-Sandoval et al. (37)	3	ARMS + drug naïve FE	36	Associative striatum, cerebellar cortex	Glu	PANSS, SIPS	NS
de la Fuente-Sandoval et al. (47)	3	FE	24	Associative striatum Cerebellar cortex	Glu Glu, Glx	PANSS PANSS	+ NS
Egerton et al. (38)	3	FE	32	ACC	Glu/Cr	PANSS negative	+
						PANSS positive	NS
				Thalamus	Glu/Cr	PANSS	NS
Ota et al. (48)	1.5	Chronic (+1 FE)	46	Inferior parietal Middle frontal	Glx Glx	PANSS positive PANSS	+ NS
Ohrmann et al. (42)	1.5	Chronic	43	ACC, DLPFC	Glx	PANSS, CDSS, CGI	NS
Tayoshi et al. (28)	3	Chronic	30	ACC, basal ganglia	Glu, Gln	PANSS	NS
Wood et al. (30)	3	Chronic	15	Dorsal, rostral cingulate	Glx	PANSS	NS
Kegeles et al. (31)	3	Chronic	32	mPFC (inclu ACC)	Glx	PANSS negative	NS
						PANSS positive	+ #
Szulc et al. (62)	1.5	Chronic	42	Frontal lobe	Glx/Cr	PANSS	+
Ohrmann et al. (41)	1.5	Chronic	39	DLPFC	Glx	PANSS	NS
Ongur et al. (50)	4	Chronic	17	ACC, POC	Gln/Glu	PANSS, MADRS, YMRS	NS
Stanley et al. (39)	1.5	FE 14wk treatment	37	DLPFC	Gln	SANS, SAPS	NS
Bartha et al. (40)	1.5	FE	10	mPFC (inclu ACC)	Glu, Gln	SANS, SAPS	NS
Theberge et al. (19)	4	FE	21	ACC, thal	Glu, Gln	SANS, SAPS	NS
Bustillo et al. (18)	4	FE Min treated	14	ACC, thal	Gln/Glu	SANS, SAPS	NS
Bustillo et al. (43)	4	Chronic	30	Whole brain slice	Glx	SANS	–

						SAPS	NS
Olbrich et al. (67)	2	FE	9	DLPFC	Glu	SANS, BPRS	–
				Hippocampus	Glu	SANS, BPRS	NS
Shirayama et al. (27)	3	Chronic	19	mPFC (inclu ACC)	Gln/Glu	SANS, BPRS	NS
Rowland et al. (44)	3	Chronic	20	mPFC, inferior parietal	Glx	SANS, BPRS	NS
Rowland et al. (46)	3	Chronic	21	ACC, CSO	Glx	SANS, BPRS	NS
Choe et al. (32)	1.5	Chronic	34	PFC	Glx/Cr	BPRS	+
Reid et al. (26)	3	Chronic	26	ACC	Glx	BPRS negative	–
						BPRS positive	NS

(Continued)

Table 1 | Continued

Reference	Field strength	Population	*n*	Brain region	Metabolite	Measure	Direction
Reid et al. (45)	3	Chronic	35	Substantia nigra	Glx/Cr	BPRS	NS
Tandon et al. (54)	1.5	Familial high risk	23	ACC, thal, caudate	Glx	WCST	NS
Rusch et al. (53)	2	FE + Chronic	29	Hippocampus	Glu	WCST	+
Shirayama et al. (27)	3	Chronic	19	mPFC (inclu ACC)	Gln/Glu	WCST, DSDT	+
						Stroop, VF, IGT	NS
Ohrmann et al. (42)	1.5	Chronic	43	ACC	Glx	WCST	–
				ACC, DLPFC	Glx	AVLT	NS
Ohrmann et al. (55)	4	FE + Chronic	35	DLPFC	Glx	AVLT	–
Kegeles et al. (31)	3	Chronic	32	mPFC (inclu ACC)	Glx	N-back	NS
Reid et al. (26)	3	Chronic	26	ACC	Glx	RBANS	NS
Reid et al. (45)	3	Chronic	35	Substantia nigra	Glx/Cr	RBANS	NS
Rowland et al. (44)	3	Chronic	20	mPFC, inferior parietal	Glx	RBANS	NS
Rowland et al. (46)	3	Chronic	21	ACC, CSO	Glx	RBANS	NS
Bustillo et al. (43)	4	Chronic	30	Whole brain slice	Glx	Combined neuropsych	–
Yoo et al. (36)	1.5	GHR	22	ACC, DLPFC, thal	Glx	GAF	NS
Tibbo et al. (59)	3	GHR	20	mPFC	Glx/Cr	GAF	–
Egerton et al. (38)	3	FE	32	ACC	Glu/Cr	GAF	+
Egerton et al. (38)	3	FE	32	Thalamus	Glu/Cr	GAF	NS
Shirayama et al. (27)	3	Chronic	19	mPFC (inclu ACC)	Gln/Glu	GAF	NS
Tebartz van Elst et al. (60)	2	Chronic	21	DLPFC	Glu	GAS	+

Aoyama et al. (34)	4	Chronic	17	Thalamus	Total Gln + Glu	LSPR	–

 # Did not survive correction for the six comparisons (PANSS total, positive, and negative symptom subscales in two regions for each neurochemical).

Index: + denotes a positive relationship where higher levels of glutamate were associated with greater symptom severity or worse overall functioning; − denotes a negative relationship where higher levels of glutamate were associated with lesser symptom severity or better overall functioning. NS, not significant; ACC, anterior cingulate cortex; POC, parieto-occipital cortex; DLPFC, dorsolateral prefrontal cortex; MPFC, medial prefrontal cortex;Thal, thalamus; CSO, centrum semiovale; GHR, genetic high risk; RBANS, repeatable battery for the assessment of neuropsychological status; WCST, Wisconsin card sorting test; DSDT, the digit span distraction test;TMT, trail making test; IGT, Iowa gambling task; VF, verbal fluency; AVLT, auditory verbal learning test; BPRS, brief psychiatric rating scale; GAF, global assessment of functioning; CGI, clinical global impression scale; GAS, global assessment scale; SIPS, structured interview for prodromal symptoms; CSS, Chapman schizotypy scales; CDSS, Calgary Depression Scale for Schizophrenia; LSPR, life skills profile rating.

psychosis (18, 37, 39, 40), or chronic schizophrenia (27, 30, 31, 41, 44, 45, 48). A general consideration of studies investigating relationships between glutamate markers and positive and negative symptoms is the scale used to score symptom severity. As detailed in **Table 1**, scores on a number of scales have been used and while these scales are highly correlated there are also some important differences in the clinical items included (51). As most studies examining relationships between brain glutamate measures and symptoms have relied on *post hoc* correlational analysis, it is of note that additional items are included on the SAPS/SANS compared to the PANSS, which in turn has additional items compared to the BPRS. Therefore use of the SAPS/SANS may be preferable as they provide a more detailed assessment of symptoms.

RELATIONSHIPS BETWEEN GLUTAMATE MEASURES AND COGNITIVE DYSFUNCTION

Relatively few studies have investigated relationships between glutamate measures and cognitive dysfunction in schizophrenia. The most commonly investigated task has been the Wisconsin card sort test (WCST): in schizophrenia, deficits on this task are associated with abnormal activation in the ACC and DLPFC (52). In a study of 19 patients with chronic schizophrenia at 3T, poor performance on the WCST was associated with higher Gln/Glu ratios in the mPFC (including the ACC) (27). In contrast, a larger study of 43 patients with chronic schizophrenia at 1.5T found that ACC, but not DLPFC, Glx levels were positively associated with WCST learning potential (42). In first-episode psychosis, an association between hippocampal, but not DLPFC, glutamate, and WCST errors was reported (53), and in a small sample of 16 genetic high risk individuals, no correlations between WCST performance and Glx levels in the caudate, ACC, or thalamus were detected (54).

Other tasks investigated include the Stroop, digit span distractibility test, auditory verbal learning test (AVLT), N-back task, Iowa gambling task and verbal fluency test (**Table 1**). Of these, there are reports of a positive association between mPFC Gln/Glu ratio and impairments on the digit span distraction test, which probes short-term memory and selective attention (27), and of DLPFC Glx and verbal learning and memory on the AVLT (55). Overall, the findings have been inconsistent and further studies with larger sample sizes are required. While the above studies used a voxel of interest method, using whole brain slice proton echo planar spectroscopy at 4T in 30 patients Bustillo et al. (43) detected a positive correlation between general cognitive performance in schizophrenia and Glx. Furthermore, subsequent path analyses suggested that the relationship between glutamate and cognitive performance may be associated with negative symptoms and unemployment (43).

The relationship between glutamate and cognition in schizophrenia has been further investigated by combining 1H-MRS with functional magnetic resonance imaging (fMRI) of the blood oxygen level dependent (BOLD) response to measure changes in regional brain activation as participants perform cognitive tasks. In subjects at clinical high risk of developing psychosis, reductions in thalamic glutamate were correlated with an elevated BOLD response in the prefrontal cortex during a verbal fluency task, whereas the converse association was observed in controls (56). In a study by Valli et al. (57), medial temporal lobe glutamate levels were correlated with hippocampal BOLD response during an episodic memory task in controls, whereas this coupling was absent in clinical high risk subjects (57). Finally one study showed that hippocampal Glx was related to inferior frontal gyrus activation during episodic memory in controls, and suggested that the absence of this positive coupling in medicated schizophrenia patients may underlie episodic memory deficits, reflecting the results seen in Valli et al. (57, 58). Further investigation of the relationships between regional levels of glutamatergic metabolites and abnormalities in regional brain activation during cognitive tasks may provide a more sensitive means to characterize the relationship between glutamate and cognition in schizophrenia.

RELATIONSHIPS BETWEEN GLUTAMATE MEASURES AND SOCIAL AND OCCUPATIONAL FUNCTIONING

Several studies have also reported correlations between brain glutamate levels and overall level of social and occupational functioning (**Table 1**). In genetically high risk subjects, lower levels of Glx/Cr in the mPFC were associated with lower levels of overall functioning (59). A longitudinal study at 4T showed that loss of glutamate and glutamine in the thalamus, but not the ACC, over 7 years since first presentation correlated with impaired social functioning (34). In contrast, in first-episode psychosis higher levels of glutamate in the ACC, but not the thalamus, were associated with worse overall functioning (38), and in chronic schizophrenia higher DLPFC glutamate levels were also associated with worse overall functioning (60). Other studies have found no association between glutamate measures and overall functioning in chronic schizophrenia (27) or genetic high risk subjects (36).

SUMMARY AND FUTURE DIRECTIONS

1H-MRS studies relating regional glutamate measures to symptoms in schizophrenia have produced inconsistent findings. There may be several methodological reasons for this, including differences in the brain region investigated, and between samples such as medication, illness stage, and symptom severity. The use of sub-optimal technical approaches such as low field strengths together with small sample sizes may also underlie the conflicting findings. A recent study at 4T indicates that elevations in the ratio of Gln/Glu are present in schizophrenia patients, consistent with elevated glutamatergic neurotransmission (18). However the majority of 1H-MRS studies in schizophrenia use field strengths <4T which cannot reliably measure glutamine, and glutamate and Glx measures cannot be specifically attributed to glutamate neurotransmission (17).

The increasing availability of higher field strength scanners may resolve some of the apparent in consistencies in the literature.

The majority of studies that have investigated relationships between glutamate levels and symptom severity have applied correlational analysis, usually *post hoc* to the main study findings. A few studies have directly compared glutamatergic metabolite levels in patient groups on the basis of symptom severity, specifically those who were or were not currently experiencing symptom exacerbation (48), were or were not in symptomatic remission following initial treatment (38) or did or did not have treatment resistant illness (49). All found higher levels of glutamate or Glx in the more symptomatic patient group.

The field would benefit from further studies pre-selecting groups of patients who differ in severity of negative symptoms or cognitive impairment, or longitudinal studies comparing within-subjects glutamate levels during periods of illness stability compared to relapse. Studies of the relationships between cognitive dysfunction and glutamate in schizophrenia may benefit from combination with fMRI to determine the efficiency of glutamate in supporting networks that subserve cognitive function. To date there are only a handful of published studies of this type. Finally, relationships between glutamate levels and symptom severity may be indirect; for example Stone et al. showed that hippocampal glutamate may interact with striatal dopamine to determine risk of psychosis (61), and a path analysis has suggested that negative symptoms may be secondary to poor cognition associated with low brain Glx (43).

One question is whether baseline levels of glutamate, glutamine, or Glx are predictive of subsequent outcome or response to treatment in schizophrenia. A recent study showed that elevated frontal Glx at baseline was associated with poor response after 4 weeks of antipsychotic treatment (62). This is consistent with the above findings of glutamatergic elevations in patients with schizophrenia whose symptoms have not responded well to treatment (38, 49).

The suggestion that symptoms that do not respond well to conventional antipsychotic treatment may have a glutamatergic basis (38, 49) warrants further investigation, as compounds that target the glutamatergic system may have particular efficacy in these patients. Meta-analyses conclude that of the agents which may improve NMDA receptor-mediated neurotransmission, the NMDAR co-agonist d-serine and the glycine transporter type 1 inhibitor sarcosine reduce total and negative symptoms as an adjuvant to antipsychotic medication (63, 64). Lamotrigine, which may inhibit glutamate release, blocks the psychomimetic effects of ketamine in healthy volunteers (65) and small trials of lamotrigine in chronic, often treatment-resistant or clozapine-treated patients found that it is beneficial in reducing symptoms (63, 66). Further work is required to determine the relationship between regional glutamate concentrations and the expression of symptoms at different stages of psychotic illness. This area may benefit from meta-analyses of the previously published findings and from new studies selecting patient samples *a priori* on the basis of symptom severity.

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2.2. Nature of Glutamate Alterations in Schizophrenia: A Meta-Analysis of Proton Magnetic Resonance Spectroscopy Studies

Kate Merritt

Alice Egerton

Matthew J. Kempton

Matthew J. Taylor

Philip K. McGuire

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Original Investigation | META-ANALYSIS

Nature of Glutamate Alterations in Schizophrenia
A Meta-analysis of Proton Magnetic Resonance Spectroscopy Studies

Kate Merritt, MSc; Alice Egerton, PhD; Matthew J. Kempton, PhD; Matthew J. Taylor, DPhil; Philip K. McGuire, FMedSci

IMPORTANCE Alterations in glutamatergic neurotransmission may be fundamental to the pathophysiology of schizophrenia, and the glutamatergic system is a target for novel therapeutic interventions in the disorder.

OBJECTIVE To investigate the nature of brain glutamate alterations in schizophrenia by conducting a meta-analysis of glutamate proton magnetic resonance (MRS) spectroscopy studies.

DATA SOURCES The MEDLINE database was searched for studies published from January 1, 1980, to April 1, 2015. Search terms included *magnetic resonance spectroscopy*, *schizophrenia*, *psychosis*, *clinical* or *genetic high risk*, and *schizoaffective*. Inclusion criteria were single voxel 1H-MRS studies reporting glutamate, glutamine or Glx values for a patient or risk group in comparison to a healthy volunteer group.

STUDY SELECTION Fifty-nine studies were identified, which included 1686 patients and 1451 healthy individuals serving as controls.

DATA EXTRACTION AND SYNTHESIS A random-effects, inverse-weighted variance model was used to calculate the pooled effect size. Mean values were extracted and verified independently. Effect sizes were determined for glutamate, glutamine, and Glx in brain regions that had been examined in at least 3 different studies. A secondary analysis grouped studies into those examining patients at different stages of illness (high risk, first-episode psychosis, or chronic schizophrenia). Effects of age, antipsychotic dose, and symptom severity were determined using meta-regression.

RESULTS In schizophrenia, there were significant elevations in glutamate in the basal ganglia (Hedges g = 0.63; 95% CI, 0.15-1.11), glutamine in the thalamus (g = 0.56; 95% CI, 0.02-1.09), and Glx in the basal ganglia (g = 0.39; 95% CI, 0.09-0.70) and medial temporal lobe (g = 0.32; 95% CI, 0.12-0.52). No region showed a reduction in glutamate metabolites in schizophrenia. Secondary analyses revealed that elevated medial frontal Glx levels were evident in individuals at high risk for schizophrenia (g = 0.26; 95% CI, 0.05-0.46) but not in those with first-episode psychosis or chronic schizophrenia, whereas elevated Glx in the medial temporal lobe was seen with chronic schizophrenia (g = 0.40; 95% CI, 0.08-0.71) but not in the high-risk or first-episode groups. Meta-regression found no association with age, symptom severity, or antipsychotic dose.

CONCLUSIONS AND RELEVANCE Schizophrenia is associated with elevations in glutamatergic metabolites across several brain regions. This finding supports the hypothesis that schizophrenia is associated with excess glutamatergic neurotransmission in several limbic areas and further indicates that compounds that reduce glutamatergic transmission may have therapeutic potential.

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Editorial

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Author Affiliations: Psychosis Studies Department, Institute of Psychiatry, Psychology, and Neuroscience, King's College London, London, England.

Corresponding Author: Kate Merritt, MSc, Psychosis Studies Department, Institute of Psychiatry, Psychology, and Neuroscience, King's College London, London SE5 8AF, England (kate.merritt@kcl.ac.uk).

Several lines of evidence have implicated alterations of the glutamatergic system in the cause of schizophrenia. The *N*-methyl-D-aspartate receptor (NMDAR) hypofunction model[1] proposes that schizophrenia is related to dysfunction of NMDARs on parvalbumin-containing γ-aminobutyric acid-ergic interneurons, leading to excess glutamate release.[2] Administration of NMDAR antagonists, such as ketamine, induce a psychotic state in healthy volunteers and exacerbate psychotic symptoms in patients with schizophrenia.[3] More recently, an autoimmune disorder associated with autoantibodies to the NMDARs has been associated with psychotic symptoms, and NMDAR autoantibodies may be evident in a small proportion of patients with schizophrenia.[4] Several genes associated with schizophrenia code for proteins involved in glutamatergic neurotransmission.[5] The main technique for assessing central glutamate function in man in vivo is proton magnetic resonance spectroscopy (MRS).

Depending on the proton MRS approach and field strength, both glutamate and the glutamate metabolite, glutamine, may be reported separately or in combination (Glx).[6] Glutamatergic metabolites are usually measured in a predetermined voxel of interest. Concentration estimates reflect both intracellular and extracellular glutamate and glutamate involved in metabolism as well as in neurotransmission.[7] Over the past 2 decades, several studies have used proton MRS to investigate regional glutamate concentrations in patients with schizophrenia compared with those in healthy volunteers. However, findings have not been consistent across studies, including reports of elevations,[8-26] no differences,[27-51] and reductions[52-63] in the patient group across a variety of brain regions. These differences may relate to regional effects, proton MRS methodologic differences, stage[24,57-59] or severity[64] of illness, or treatment effects.[17,20] The first meta-analysis[65] of these studies reported decreases in glutamate and increases in glutamine in the medial frontal cortex of patients compared with controls. The total number of publications has more than doubled since the first meta-analysis, which included studies up until 2011. These more recent reports include studies on regions of interest that had previously been examined too infrequently to be included in a meta-analysis. Moreover, the field now includes substantial numbers of studies in individuals at high risk (HR) for schizophrenia and those with first-episode psychosis (FEP), in addition to studies in patients with chronic schizophrenia, permitting separate meta-analyses of these different groups.

The primary aim of this study was to conduct an updated case-control meta-analysis of all published reports of regional glutamatergic measures in those at HR for schizophrenia, with FEP, and with schizophrenia. The second aim was to conduct case-control meta-analyses in clinical subgroups separately (HR, FEP, and chronic schizophrenia [referred to as *schizophrenia* hereinafter]). The third aim was to assess the influences of age, symptom severity, and antipsychotic treatment.

The first hypothesis of the study was that, on the basis of preclinical schizophrenia models showing increases in glutamatergic transmission,[66] glutamatergic metabolites would be increased in cases compared with controls. The second hypothesis was that there would be higher glutamatergic metabolite concentrations in FEP and HR individuals compared with those who had schizophrenia, in line with previous studies comparing patient groups.[23,24,57-59] The third hypothesis was that glutamate and glutamine levels would become lower with antipsychotic treatment[17,20,21] as well as with age in cases relative to controls,[65] but that symptom severity would be associated with higher glutamatergic metabolite concentrations.[64]

Key Points

Question What is the nature of glutamate alterations in schizophrenia as revealed by studies using proton magnetic resonance spectroscopy?

Findings This meta-analysis evaluated 59 studies reporting on regional glutamate, glutamine, or their combined Glx signals. There were significant elevations in glutamate in the basal ganglia, glutamine in the thalamus, and Glx in the basal ganglia and medial temporal lobe but no associations with age, symptom severity, or antipsychotic medication dose.

Meaning Schizophrenia is associated with elevations in glutamate-related metabolites across several brain regions consistent with the hypothesis that there is excess glutamatergic neurotransmission in this condition.

Methods

Study Selection

The MEDLINE database was searched to identify journal articles published between January 1, 1980, and April 1, 2015, using the following search terms: *MRS* or *magnetic resonance spectroscopy* and (1) *schizophrenia* or (2) *psychosis* or (3) *UHR* or (4) *ARMS* or (5) *ultra high risk* or (6) *clinical high risk* or (7) *genetic high risk* or (8) *prodrom** or (9) *schizoaffective*. All single-voxel proton MRS studies reporting glutamate, glutamine, or Glx values for a patient or risk group in comparison with a healthy volunteer group were included in the analysis. In the case of longitudinal studies,[17,43,52] only the values given for the first time point were included. If the same sample or partially overlapping samples were included in more than 1 report, data from the study with the largest sample were included (References 9, 10, 17, 24, 38, 43, 45, 52, 54, 62).

Meta-analysis

Mean values of proton MRS glutamate, glutamine, or Glx concentrations were extracted by one of us (K.M.) and verified by another (A.E.) independently and categorized into the following brain regions of interest: (1) medial frontal cortex, including studies with voxels in the medial prefrontal cortex and in the anterior cingulate cortex since these voxels often spatially overlap; (2) dorsolateral prefrontal cortex (DLPFC); (3) frontal white matter; (4) thalamus; (5) medial temporal lobe (MTL) (including hippocampus); (6) basal ganglia (including caudate, putamen, and globus pallidus); and (7) cerebellum.

Only analyses for which at least 3 independent data sets were available were included. When more than 1 clinical group was reported in a single study, the values were treated as independent data sets and the number of healthy volunteers was adjusted by dividing by the number of clinical groups. When data were reported bilaterally, only those for the left hemisphere were included because the left hemisphere was examined in most studies.

The ability of proton MRS to resolve the overlapping resonances of glutamate and glutamine increases with field strength. Previous estimates[6] of the degree of contamination of glutamate and glutamine signals at different field strengths using optimized sequences indicated that it would be appropriate to include studies reporting glutamate if the data were acquired at field strengths of 3 T or above and studies reporting glutamine at 4 T or above. A secondary analysis included data acquired at all field strengths.

The proton MRS measures of glutamate, glutamine, or Glx were analyzed separately, which was accounted for by applying a Bonferroni-corrected threshold for statistical significance of $P < .017$. The effect size statistic Hedges g, which incorporates a correction for bias from small sample sizes, was calculated by subtracting the mean glutamate, glutamine, or Glx values reported in cases by the mean value reported in the control group divided by the pooled SD across groups.[67] If means or SDs were not reported, authors were contacted for this information. A Hedges g value of 0 indicates no difference between cases and controls, negative values indicate lower glutamatergic metabolite levels in cases than controls, and positive values denote higher glutamatergic metabolite levels in cases than controls.

A random-effects, inverse-weighted variance model[68] was used to calculate the pooled effect size since the studies were expected to display high heterogeneity as different correction methods and clinical samples were used. Study effect size was weighted according to sample size. Heterogeneity was measured using the I^2 value, with higher percentages denoting higher variation across studies in the meta-analysis. The meta-analysis for each brain region was performed using meta-analytical equations entered into Excel (Microsoft Corp) (http://www.depressiondatabase.org). These equations are identical to the METAN command in Stata (StataCorp LP), which is commonly used in meta-analyses publications. In terms of validation, the method has been used in parallel with Stata in previous meta-analyses[69] and produced the same results.

Effect sizes were initially calculated for all patients and controls and then for each clinical group (HR, FEP, and schizophrenia) separately. Separate analysis was also performed of patient groups (FEP and schizophrenia) since most HR individuals will not develop psychosis.

Meta-regression

To explore the relationship between glutamate, glutamine, and Glx effect sizes for each study and selected demographics or clinical variables, random effects meta-regressions were conducted using the metareg command in Stata, version 11.2 2009. The variables investigated were age; Positive and Negative Syndrome Scale (PANSS)[70] total; positive, negative, and general subscale scores; chlorpromazine-equivalent dose; and duration of illness. In studies that used the Brief Psychiatric Rating Scale, the scores were converted to PANSS scores.[71] When these measures were not reported, study authors were contacted to request the data. Publication bias was examined using the Egger regression test for regions including at least 10 studies[72] and meta-regressions of study effect size and year of publication. A leave-one-out jackknife sensitivity analysis was conducted for regions with at least 4 studies in which significant between-group differences were found.

Results

The literature search identified 59 studies, with a total of 1686 cases and 1451 controls (PRISMA flow diagram presented in eFigure 1 in the Supplement). The sample sizes ranged from 5 to 84 for cases and 4 to 81 for healthy volunteers (eTable 1 in the Supplement). Two studies[73,74] reporting multivoxel data were excluded.

Fourteen studies (References 18, 25, 26, 29, 31, 37, 49, 51-53, 56, 57, 62, 75) examined participants at HR for psychosis. Eighteen studies (References 9, 12, 17-19, 23, 24, 28, 30, 32, 33, 45, 48, 57-59, 63, 76) examined patients experiencing a first episode of psychosis (FEP), all with an onset of illness within the last 2½ years. Thirty-six studies (References 8, 10, 11, 13-16, 20-24, 27, 31, 34-36, 38-44, 46, 47, 50, 53-55, 57-61, 77) examined patients with established (chronic) schizophrenia (eResults in the Supplement provide detailed patient information).

Meta-analysis

In the medial frontal cortex, there were no significant findings for glutamate (HR group, 3; FEP group, 3; and schizophrenia group, 11), Glx (HR group, 8; FEP group, 3; and schizophrenia group, 13) or glutamine (HR group, 0; FEP group, 2; and schizophrenia group, 3) (**Table** and eFigure 2 in the Supplement). Analysis of each clinical group separately revealed higher Glx concentrations in HR individuals ($g = 0.26$; 95% CI, 0.05-0.46; $P = .01$). There were no significant between-group differences in glutamate or glutamine levels.

In the frontal white matter, there were no significant effects overall for glutamate (HR group, 1; FEP group, 1; schizophrenia group, 1; and FEP + schizophrenia group, 1) or Glx (HR group, 0; FEP group, 2; and schizophrenia group, 7) in cases compared with controls, and only 1 study[76] examined glutamine (Table). The schizophrenia group showed elevated Glx levels compared with controls ($g = 0.42$; 95% CI, 0.18-0.66; $P = .001$).

In the DLPFC, there were no significant effects for Glx in cases (HR group, 2; FEP group, 2; and schizophrenia group, 8) or in the schizophrenia group. There were insufficient data above 1.5 T for glutamate and at 4 T for glutamine in the DLPFC.

In the basal ganglia, both glutamate (HR group, 1; FEP group, 2; and schizophrenia group, 1; $g = 0.63$; 95% CI, 0.15-1.11; $P = .01$) and Glx concentrations (HR group, 4; FEP group, 3; and schizophrenia group, 2; $g = 0.39$; 95% CI, 0.09-0.70; $P = .01$) (**Figure 1** and **Figure 2**) were higher in cases than in controls. Subgroup analysis of Glx found elevations in the FEP group ($g = 0.66$; 95% CI, 0.28-1.03; $P < .001$) but not in the HR

Table. Meta-analysis Results Summary for All Patient Groups in All Brain Regions

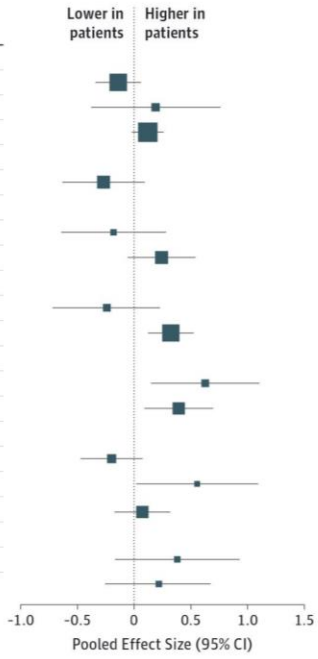
Brain Region by Metabolite	Group	Studies	Cases	Healthy Controls	Effect Size 95% CI	Effect Size P Value	Heterogeneity I^2, %	Heterogeneity P Value
Medial frontal cortex								
Glutamate	All cases (3 HR, 3 FEP, 11 SZ)	17	411	381	−0.14 (−0.34 to 0.06)	.17	39.7	.05
	HR	3	102	80	−0.09 (−0.68 to 0.50)	.77	61.3	.08
	FEP (0 medicated, 3 unmedicated)	3	34	28	−0.09 (−0.46 to 0.29)	.64	12.8	.32
	SZ (11 medicated, 0 unmedicated)	11	242	233	−0.16 (−0.44 to 0.12)	.24	18.7	.04
Glutamine	All cases (0 HR, 2 FEP, 3 SZ)	5	84	78	0.19 (−0.38 to 0.76)	.52	67.7	.02
	SZ (3 medicated, 0 unmedicated)	3	50	50	−0.19 (−0.78 to 0.40)	.53	51.7	.13
Glx	All cases (8 HR, 3 FEP, 13 SZ)	24	487	440	0.12 (−0.02 to 0.27)	.10	13.1	.28
	HR	8	203	183	0.26 (0.05 to 0.46)	.01[a]	0.0	.51
	FEP (1 medicated, 2 unmedicated)	3	49	49	0.03 (−0.37 to 0.42)	.90	0.0	.37
	SZ (12 medicated, 1 unmedicated)	13	235	208	0.02 (−0.21 to 0.24)	.89	20.5	.24
DLPFC								
Glutamate	All cases (0 HR, 0 FEP, 1 SZ)	1	NA	NA	NA	NA	NA	NA
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (2 HR, 2 FEP, 8 SZ)	12	262	184	−0.27 (−0.63 to 0.09)	.15	68.6	<.001
	SZ (7 medicated, 1 unmedicated)	8	172	132	−0.32 (−0.85 to 0.21)	.23	77.6	<.001
Frontal WM								
Glutamate	All cases (1 HR, 1 FEP, 1 SZ, 1 FEP + SZ)	4	57	48	−0.18 (−0.64 to 0.28)	.44	25.6	.26
Glutamine	All cases (0 HR, 1 FEP, 0 SZ)	1	NA	NA	NA	NA	NA	NA
Glx	All cases (0 HR, 2 FEP, 7 SZ)	9	261	135	0.24 (−0.06 to 0.54)	.11	46.2	.06
	SZ (4 medicated, 3 unmedicated)	7	200	110	0.42 (0.18 to 0.66)	.001[a]	0.0	.58
MTL								
Glutamate	All cases (3 HR, 0 FEP, 3 SZ)	6	83	91	−0.24 (−0.71 to 0.24)	.33	55.3	.05
	HR	3	47	49	−0.34 (−0.86 to 0.17)	.19	30.7	.24
	SZ (2 medicated, 1 mixed)	3	36	43	−0.08 (−1.02 to 0.86)	.87	75.0	.02
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (5 HR, 5 FEP, 8 SZ)	18	441	350	0.32 (0.12 to 0.52)	.002[a]	42.0	.03
	HR	5	112	78	0.36 (−0.14 to 0.86)	.16	56.7	.06
	FEP (4 medicated, 1 unmedicated)	5	132	94	0.12 (−0.16 to 0.40)	.39	2.2	.39
	SZ (5 medicated, 3 unmedicated)	8	197	179	0.40 (0.08 to 0.71)	.01[a]	51.3	.04
Basal ganglia								
Glutamate	All cases (1 HR, 2 FEP, 1 SZ)	4	89	83	0.63 (0.15 to 1.11)	.01[a]	57.2	.07
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (4 HR, 3 FEP, 2 SZ)	9	216	182	0.39 (0.09 to 0.70)	.01[a]	51.5	.04
	HR	4	116	102	0.23 (−0.34 to 0.80)	.43	73.7	.01
	FEP (1 medicated, 2 unmedicated)	3	59	56	0.66 (0.28 to 1.03)	<.001[a]	0.0	.99
Thalamus								
Glutamate	All cases (1 HR, 2 FEP, 1 SZ)	4	125	103	−0.20 (−0.47 to 0.08)	.16	4.5	.37
Glutamine	All cases (0 HR, 2 FEP, 1 SZ)	3	50	48	0.56 (0.02 to 1.09)	.04	40.5	.19
Glx	All cases (3 HR, 2 FEP, 2 SZ)	7	240	159	0.07 (−0.17 to 0.32)	.56	24.1	.24
	HR	3	120	101	0.16 (−0.39 to 0.71)	.57	71.7	.03
Cerebellum								
Glutamate	All cases (1 HR, 2 FEP, 0 SZ)	3	59	58	0.38 (−0.17 to 0.93)	.17	54.2	.11
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (1 HR, 2 FEP, 0 SZ)	3	59	58	0.22 (−0.25 to 0.68)	.36	37.5	.20

Abbreviations: DLPFC, dorsolateral prefrontal cortex; FEP, first-episode psychosis; Glx, combined glutamate and glutamine signal; HR, high risk; MTL, medial temporal lobe; NA, not applicable; SZ, chronic schizophrenia; WM, white matter.

[a] Results that survived multiple comparisons for each region as glutamate, glutamine, and Glx were investigated.

Figure 1. Summary Effect Sizes for Glutamatergic Differences Between Cases and Controls in Each Brain Region Examined

Area	Hedges *g* (95% CI)	I^2, %
Medial frontal cortex		
Glutamate	-0.14 (-0.34 to 0.06)	40
Glutamine	0.19 (-0.38 to 0.76)	68
Glx	0.12 (-0.02 to 0.27)	13
DLPFC		
Glx	-0.27 (-0.63 to 0.09)	69
Frontal WM		
Glutamate	-0.18 (-0.64 to 0.28)	26
Glx	0.24 (-0.06 to 0.54)	46
MTL		
Glutamate	-0.24 (-0.71 to 0.24)	55
Glx	0.32 (0.12 to 0.52)	42
Basal ganglia		
Glutamate	0.63 (0.15 to 1.11)	57
Glx	0.39 (0.09 to 0.70)	52
Thalamus		
Glutamate	-0.20 (-0.47 to 0.08)	5
Glutamine	0.56 (0.02 to 1.09)	41
Glx	0.07 (-0.17 to 0.32)	24
Cerebellum		
Glutamate	0.38 (-0.17 to 0.93)	54
Glx	0.22 (-0.25 to 0.68)	38



Negative Hedges *g* values denote lower glutamatergic metabolite concentrations in cases than controls; positive values denote higher glutamatergic metabolite concentrations in cases than controls. The size of the data markers is proportional to the total number of individuals. DLPFC indicates dorsolateral prefrontal cortex; Glx, combined glutamate and glutamine signal; MTL, medial temporal lobe; and WM, white matter.

group; there were insufficient studies in patients with schizophrenia to determine the results.

In the MTL, Glx was increased in cases compared with controls (HR group, 5; FEP group, 5; and schizophrenia group, 8; g = 0.32; 95% CI, 0.12-0.52; P = .002) (Figures 1 and 2) but not glutamate (HR group, 3; FEP group, 0; and schizophrenia group, 3). Subgroup analysis found significantly higher Glx only in the schizophrenia group (g = 0.40; 95% CI, 0.08-0.71; P = .01) (Figure 2). There were no between-group differences in glutamate levels. The same results were found after excluding patients with 22q11 deletion.[16] There were insufficient data at 4 T for glutamine.

In the thalamus, glutamine concentrations were higher in cases than controls (FEP group, 2; schizophrenia group, 1; g = 0.56; 95% CI, 0.02-1.09; P = .04) (Figure 2). There were no between-group differences in glutamate (HR group, 1; FEP group, 2; and schizophrenia group, 1) or Glx (HR group, 3; FEP group, 2; and schizophrenia group, 2). No significant effects were present for glutamate or Glx in the cerebellum (HR group, 1; FEP group, 2; and schizophrenia group, 0) (Table).

Analysis Limited to Patient Groups

When analysis was limited to patients by excluding the HR group, no additional significant findings were apparent in any region (eTable 2 in the Supplement). Elevated Glx levels in MTL (g = 0.31; 95% CI, 0.09-0.53; P = .007) and basal ganglia (g = 0.57; 95% CI, 0.26-0.88; P < .001), as well as elevated glutamine levels in the thalamus (g = 0.56; 95% CI, 0.02-1.10; P = .04), remained significant; however, glutamate in the basal ganglia (P = .08) was no longer significant.

Meta-analysis Including Studies at Low Field Strength

Meta-analyses were repeated including studies that measured glutamate or glutamine at low field strengths (<3 T for glutamate[12,23,28,32] or <4 T for glutamine (References 10, 12, 13, 16, 23, 32, 35, 55, 62, 77)). Inclusion of these studies did not change the finding of no difference in glutamate levels overall in the medial frontal region, although glutamine levels were significantly elevated in cases (HR group, 1; FEP group, 3; and schizophrenia group, 7; g = 0.35; 95% CI, 0.02-0.67; P = .04; I^2 = 63%) and in the FEP group (g = 0.84; 95% CI, 0.38-1.30; P < .001; I^2 = 0%).

In the DLPFC, there were no significant effects for glutamate (HR group, 0; FEP group, 3; and schizophrenia group, 3) or glutamine (HR group, 0; FEP group, 2; schizophrenia group, 4) in cases compared with controls. The schizophrenia group showed elevated glutamine levels in the DLPFC (g = 0.46; 95% CI, 0.06-0.86; P = .02; I^2 = 25%), which was no longer significant when 1 study[16] in patients with a 22q11 deletion was excluded. There were no between-group differences in glutamate levels.

In the MTL, glutamine levels were increased in cases compared with controls (HR group, 1; FEP group, 1; and schizophrenia group, 2; g = 0.41; 95% CI, 0.02-0.80; P = .04; I^2 = 0%). There remained no differences in glutamate for cases or separate clinical groups (HR group, 3; FEP group, 1; and schizophrenia group, 4). The same results were found after excluding patients with a 22q11 deletion.[16]

All studies of glutamate in the frontal white matter, basal ganglia, thalamus, and cerebellum were above 1.5 T. All studies of glutamine in the thalamus were at 4 T.

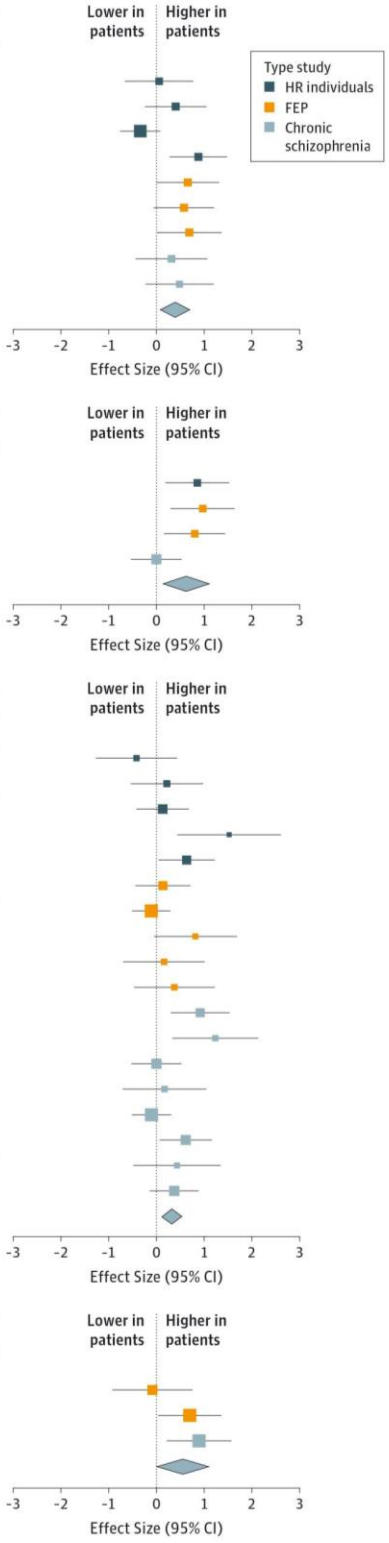
Figure 2. Study Effect Sizes in Brain Regions Showing Significant Glutamatergic Differences Between Cases and Controls

Source	Hedges *g* (95% CI)
Basal ganglia: Glx	
Block et al,[31] 2000	0.06
de la Fuente-Sandoval et al,[18] 2011	0.40
Keshavan et al,[37] 2009	-0.34
Tandon et al,[25] 2013	0.88
de la Fuente-Sandoval et al,[18] 2011	0.66
de la Fuente-Sandoval et al,[17] 2013	0.63
Goto et al,[19] 2012	0.69
Block et al,[31] 2000	0.31
Yamasue et al,[50] 2003	0.49
Summary: $g = 0.39$ (95% CI, 0.09-0.70); $I^2 = 52\%$	

Source	Hedges *g* (95% CI)
Basal ganglia: glutamate	
de la Fuente-Sandoval et al,[18] 2011	0.86
de la Fuente-Sandoval et al,[18] 2011	0.97
de la Fuente-Sandoval et al,[17] 2013	0.80
Tayoshi et al,[55] 2009	0.00
Summary: $g = 0.63$ (95% CI, 0.15-1.11); $I^2 = 57\%$	

Source	Hedges *g* (95% CI)
Medial temporal lobe: Glx	
Capizzano et al,[75] 2011	-0.42
Capizzano et al,[75] 2011	0.22
Stone et al,[62] 2009	0.13
Wood et al,[49] 2010	1.52
Wood et al,[49] 2010	0.63
Galińska et al,[30] 2009	0.14
Hasan et al,[33] 2014	-0.11
Szulc et al,[24] 2004	0.82
Wood et al,[48] 2008	0.16
Wood et al,[48] 2008	0.38
Chang et al,[14] 2007	0.92
da Silva Alves et al,[16] 2011	1.23
Hutcheson et al,[34] 2012	0.00
Kegeles et al,[36] 2000	0.17
Kraguljac et al,[38] 2012	-0.10
Kraguljac et al,[21] 2013	0.61
Szulc et al,[24] 2004	0.43
Szulc et al,[43] 2011	0.37
Summary: $g = 0.32$ (95% CI, 0.12-0.52); $I^2 = 42\%$	

Source	Hedges *g* (95% CI)
Thalamus: glutamine	
Bustillo et al,[76] 2010	-0.08
Théberge et al,[9] 2002	0.70
Théberge et al,[54] 2003	0.89
Summary: $g = 0.56$ (95% CI, 0.02-1.09); $I^2 = 41\%$	



Each data marker represents a study, and the size of the data marker is proportional to the total number of individuals in that study. The summary effect size for each brain region is denoted by a blue diamond. FEP indicates first-episode psychosis; Glx, combined glutamate and glutamine signal; and HR, high risk.

Heterogeneity

Significant heterogeneity was found in numerous groups for all regions (Table), which justifies the use of a random-effects model to combine the effect sizes. Heterogeneity may result from methodologic differences (eTable 3 in the Supplement). Meta-regression analysis investigated possible sources of heterogeneity.

Meta-regression

The clinical and demographic measures that were available from each study for meta-regression are presented in eTable 1 in the Supplement. In all brain regions, there were no significant correlations between the study effect sizes for glutamate, glutamine, or Glx and the mean PANSS subscale scores, chlorpromazine-equivalent dose, and duration of psychotic illness of patients with FEP or schizophrenia.

In all brain regions, there was no association between the mean age of the patients and the effect size for glutamate, glutamine, or Glx.

Small-Study Bias

Small-study bias, which could reflect publication bias, was evident for reports of Glx in the medial frontal region (Egger test, $P = .01$) and Glx in the basal ganglia ($P = .04$). The year of publication was not significantly associated with metabolite reports in any brain region.

Sensitivity Analysis

Leave-1-out sensitivity analysis showed that significant results were generally robust. Significant differences did not remain in 2 of 3 tests for cases of glutamine in the thalamus, indicating an unreliable result.

Discussion

The number of publications reporting proton MRS measures of brain glutamate, glutamine, or Glx in schizophrenia has more than doubled since the last meta-analysis.[65] In addition to analyzing data from a large number of new studies, we were able to include findings from brain regions precluded from previous meta-analysis in the present study.

We found significant differences in glutamatergic metabolites across several cortical and subcortical regions in cases compared with controls. Although the nature of the findings varied depending on the patient subgroup and brain region, all of the significant findings reflected elevations in glutamatergic metabolites in patients and HR individuals. This finding is consistent with data from animal models of schizophrenia that propose an increase in glutamatergic activity resulting from NMDAR hypofunction.[66] The finding is also consistent with human studies of NMDAR hypofunction that show increases in both glutamate and glutamine concentrations in the cortex following ketamine administration to healthy volunteers.

The HR, FEP, and schizophrenia groups had higher glutamate and Glx levels in the basal ganglia, higher glutamine levels in the thalamus, and higher Glx levels in the MTL. In contrast, there were generally no significant findings in the DLPFC or cerebellum, and significant findings in the medial frontal cortex and frontal white matter were observed only in specific patient subgroups. Preclinical models propose that glutamatergic overactivity in hippocampal areas drive excessive subcortical dopamine release via polysynaptic glutamatergic projections to the striatum. Likewise, abnormalities in striatal glutamate may influence striatal dopaminergic signaling since glutamate in the basal ganglia modulates tonic dopamine release presynaptically via NMDARs. Finally, the thalamus receives efferent input from the striatum,[78] and NMDAR antagonism in the thalamus causes cortical neurotoxic injury via corticothalamic loops.

In all regions except the basal ganglia, the HR, FEP, and schizophrenia groups had significant elevations in the Glx or glutamine signal rather than the glutamate signal, which may partially reflect the greater number of studies reporting on Glx rather than glutamate or glutamine separately. Although the glutamate signal accounts for most (80%-90%) of the Glx signal at field strengths of 1.5 T to 3 T,[6] it is possible that Glx level elevations could be driven by increases in glutamine rather than glutamate levels. Following neurotransmission, glutamate is converted to glutamine in astrocytes for recycling. Elevations in glutamine levels may thus reflect increases in glutamatergic synaptic activity. The previous meta-analysis[27] reported reduced medial frontal glutamate but elevated glutamine levels in schizophrenia that were not detected in the present analysis. This discrepancy may reflect improved methods in more recent studies since more studies acquired data at higher field strengths, correct for voxel cerebrospinal fluid, and specify more conservative thresholds for the acceptability of metabolite fitting (eTable 3 in the Supplement).

There was some evidence that the regional degree of glutamatergic elevation may be sensitive to illness stage. The Glx level elevations in the medial frontal cortex were apparent in HR individuals but not in those with schizophrenia. Similarly, medial frontal glutamine levels were elevated in 2 studies[9,76] of patients with FEP, but no differences were seen in patients with schizophrenia. Conversely, MTL Glx levels were elevated in individuals with schizophrenia but not in the FEP or HR groups. One interpretation of these findings is that the regional pattern of glutamatergic abnormalities progress with the clinical course of the disorder or show differential responses to antipsychotic treatment. Most HR individuals will not develop psychosis and were not receiving antipsychotic medication; exclusion of this group generally resulted in similar effect sizes. This observation suggests that inclusion of HR groups did not dilute the findings in patients and that the same pattern of glutamatergic-level elevation is apparent in individuals at HR for psychosis. Few studies have directly compared different patient groups[23,24,57-59] or repeatedly assessed glutamatergic metabolites over long periods.[79] Interpretation was limited because there were insufficient data to analyze all glutamatergic measures for each patient group separately in every region.

The meta-regression did not find support for the hypothesis that glutamatergic metabolite concentrations in patients vary in association with age, antipsychotic treatment, or symp-

tom severity. The latter may be relevant to the interpretation of cross-sectional studies comparing regional glutamatergic measures in association with antipsychotic treatment response[8] because this finding suggests that such differences may not simply involve group differences in symptom severity. Detailed information on antipsychotic treatment was available in few data sets, and mean chlorpromazine-equivalent doses may not account for medication adherence and do not discriminate between the effects of different antipsychotic medications. Longitudinal MRS studies have not found effects of antipsychotic treatment on medial frontal glutamatergic measures,[76,79] although medication effects in the striatum have been reported.[17] One previous study[20] reported higher medial frontal Glx in patients not receiving vs receiving medications. The meta-analysis found higher medial frontal glutamine (but not glutamate or Glx) levels in patients with FEP, all of whom were not receiving medications.

One limitation of the present meta-analysis is that, when clinical groups were analyzed separately, the number of studies per group for some regions was small. We conducted the meta-analysis when at least 3 independent data sets were available; however, findings based on a low number of data sets should be considered preliminary and are presented to stimulate further research. Increases in Glx levels in the medial frontal cortex in HR individuals and increases in MTL Glx levels in patients with schizophrenia were reported by relatively large numbers of studies; thus, these investigations represent the most robust of the findings in patient subgroups.

Our HR category included studies of people at increased familial risk for schizophrenia as well as those showing subclinical signs of psychosis since there were too few studies to permit separate meta-analyses of each group. The risk of psychosis differs between these groups, and this heterogeneity may explain why we did not find elevated Glx levels in the basal ganglia and lower glutamate levels in the thalamus that have been reported[52] in clinical HR individuals. In addition, glutamate may be increased only in HR persons who will later develop psychosis or show poorer outcomes.[52]

The resonance frequencies of glutamate and glutamine significantly overlap at 1.5 T, whereas glutamate can be largely resolved at 3 T, and field strengths of 4 T or more are needed to measure glutamine accurately.[6] For glutamine reports, a sufficient number of studies were performed at 4 T only in the thalamus. Given that glutamine may provide a measure of glutamate turnover and our general finding of increased glutamine or Glx rather than glutamate in patients, additional studies at higher field strengths optimized for glutamine resolution should be a priority. Another limitation of this study is that there was much variability in data acquisition and analysis methods (eTable 3 in the Supplement), which will affect data quality. Full reporting of such information in future studies will help to address sources of heterogeneity in subsequent meta-analyses.

Use of proton MRS provides a measure of total concentrations within the voxel studied and thus does not infer the functional significance of the metabolites measured. However, glutamine can act as an indirect measure of neurotransmitter glutamate turnover since 80% of glutamine is used for glutamate neurotransmitter cycling.[7]

Conclusions

This meta-analysis indicates that schizophrenia is associated with glutamatergic-level elevations in several brain regions. These findings further support the idea that pharmacologic compounds that can reduce glutamatergic neurotransmission may have therapeutic potential.

ARTICLE INFORMATION

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2.2.1. Supplementary Content

Supplementary Online Content

Merritt K, Egerton A, Kempton MJ, Taylor MJ, McGuire PK. Nature of glutamate alterations in
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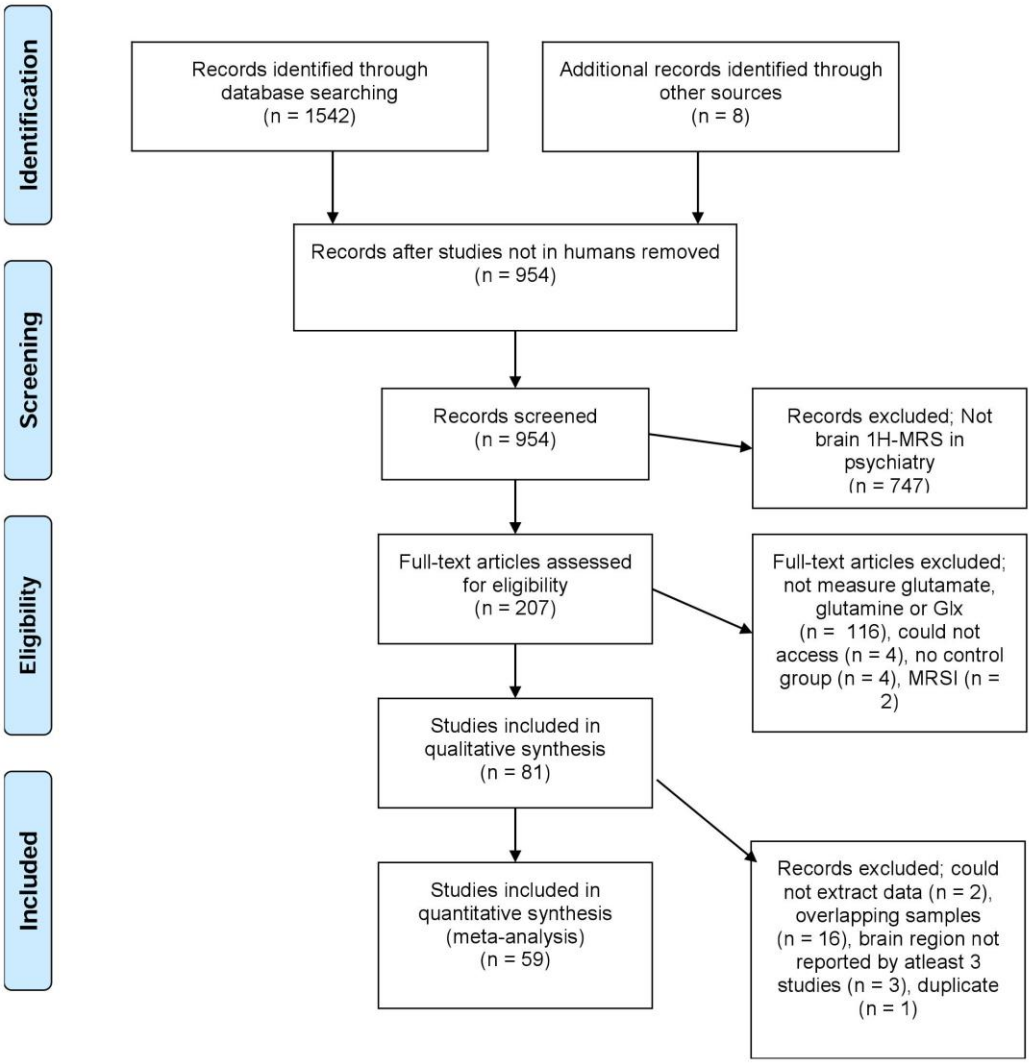
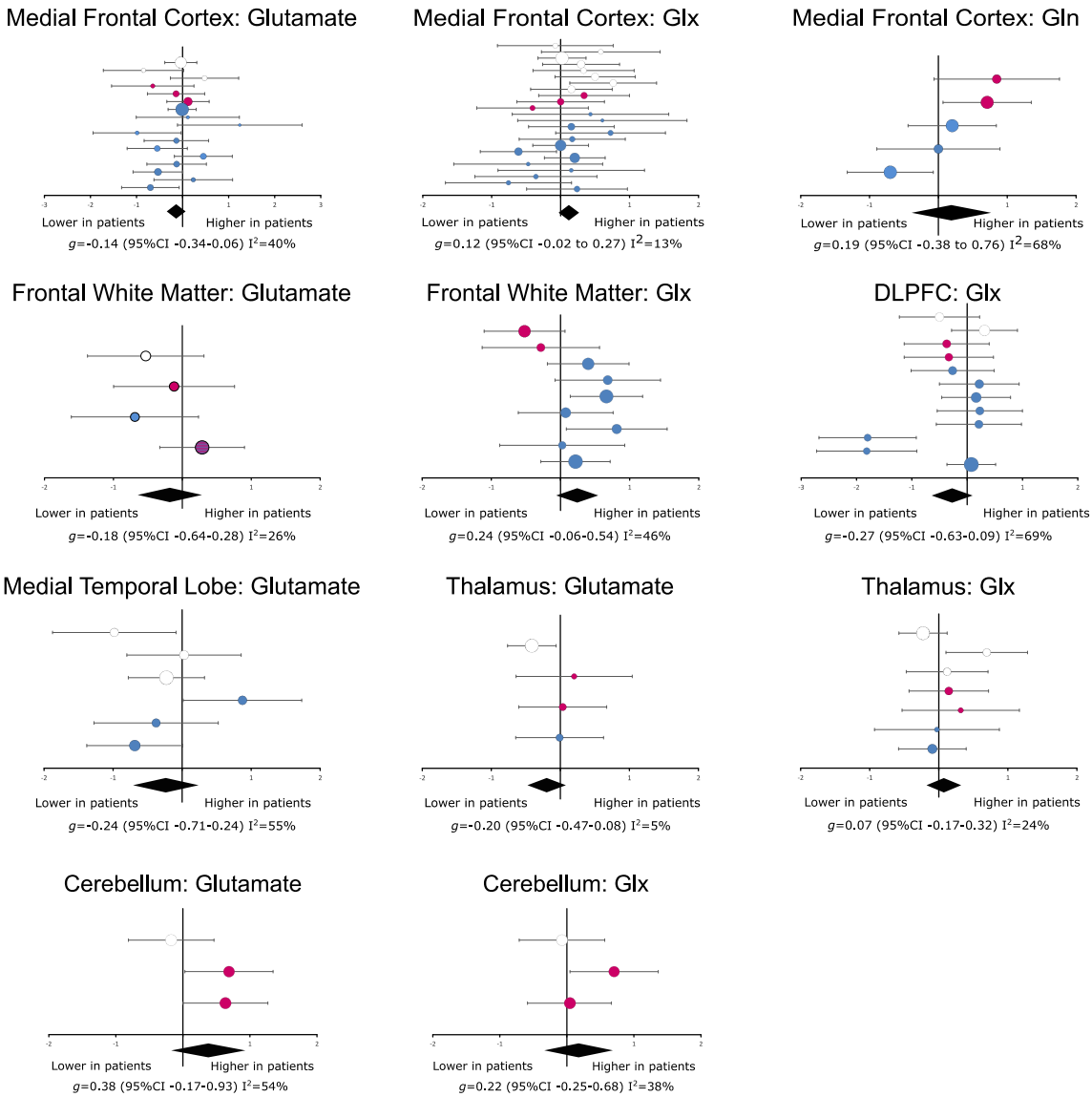
eFigure 1. PRISMA Flow Diagram of Literature Search and Study Selection
eFigure 2. Study Effect Sizes of Glutamatergic Differences Between Cases and Controls
eTable 1. List of Studies Included in the Meta-analysis and Available Measures
eTable 2. Meta-analysis Results Summary for Patients With Schizophrenia (First-Episode Psychosis and
Chronic Schizophrenia) in All Brain Regions
eTable 3. List of Studies Included in the Meta-analysis and Methodological Variables
eResults. Patient Details

This supplementary material has been provided by the authors to give readers additional information about
their work.



eFigure1: PRISMA flow diagram of literature search and study selection.



eFigure2: Study effect sizes of glutamatergic differences between cases and controls.

Shown are Hedges g effect size and 95% confidence intervals (95%CI) for each study. Each circle represents a study, and circle size is proportional to the number of patients and controls. White circles represent studies of high risk subjects, pink circles represent studies of first episode psychosis, blue circles represent studies of patients with chronic schizophrenia. The summary effect size for each brain region is denoted by a diamond. DLPFC; Dorsolateral prefrontal cortex, Gln; Glutamine.

eTable 1. List of Studies Included in the Meta-analysis and Available Measures

HR; high risk, FEP; first episode psychosis. Medial Frontal Cortex; medial prefrontal cortex + anterior cingulate cortex, DLPFC; dorsolateral prefrontal cortex, frontal WM; frontal white matter. L; Left, R; Right, B; Bilateral. Glu; glutamate, Gln; glutamine. PANSS; Positive and Negative Syndrome Scale, (* indicates PANSS values converted from BPRS scores). CPZ; Chlorpromazine equivalent dose. For "Medicated": ticks (✓) represent studies of currently medicated subjects, crosses (×) represent currently unmedicated subjects, and "both" represent a mixed sample of both medicated and unmedicated patients.

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
Medial Frontal Cortex	L	Bartha 1997	FEP	10	10	Glu	-0.42	1.5	×	24.4	-	-	-	-	-	×
						Gln	1.11									
	B	Bustillo 2010	FEP	14	8	Glu	-0.65	4	×	27.2	-	-	-	-	-	2.5
						Gln	0.85									
	B	Bustillo 2013	Chronic	84	81	Glu	-0.01	3	✓	36.7	62.3	15.1	14.5	30.5	×	×
				72	76	Gln	0.43									
	B	Capizzano 2011	HR (1st degree relative)	12	10	Glx	0.58	3	-	19.3	-	-	-	-	-	-
	B		HR (2nd degree relative)	12	10	Glx	-0.08	3	-	19.5	-	-	-	-	-	-
	B	Demjaha 2013	Chronic responder	8	5	Glu	0.11	3	✓	45.6	50.1	12.1	13.9	24.1	289.4	15.8
						Glx	0.43									
			Chronic treatment resistant	6	5	Glu	1.24	3	✓	42.8	103.7	26.0	27.2	50.5	358.8	13.3
						Glx	0.61									
	B	Egerton 2014	High risk	75	55	Glu	-0.04	3	-	23.3	-	-	-	-	-	-
						Glx	0.02									
	B	Goto 2012	FEP	18	18	Glx	0.34	3	✓	31.0	68.1	15.9	17.2	34.2	293.4	0.6
	L	Jessen 2013	Chronic	20	20	Gln	0.37	3	✓	34.5	51.0	10.8	13.9	26.4	×	7.2
						Glx	0.16									
	B	Kegeles 2012	Chronic unmedicated	16	11	Glx	0.72	3	×	32.0	71.0	×	×	×	-	7.0
			Chronic medicated	16	11	Glx	0.17	3	✓	32.0	57.0	×	×	×	×	9.0
	B	Kraguljac 2012	Chronic	48	46	Glx	0.00	3	✓	38.0	58.0*	×	×	×	×	16.4

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
Medial Frontal Cortex	B	Lutkenhoff 2010	High risk	12	11	Glu	-0.85	3	-	49.5	-	-	-	-	-	-
			Chronic	9	11	Glu	-0.99	3	✓	48.8	×	×	×	×	×	27.4
	B	Marsman 2014	Chronic	14	18	Glu	-0.13	7	✓	27.6	53.1	12.7	12.9	27.5	×	6.5
	B	Natsubori 2014	High risk	24	26	Glx	0.30	3	-	21.7	-	-	-	-	-	-
			FEP	19	19	Glx	0.00	3	×	25.4	71.3	16.0	18.8	36.4	-	0.7
			Chronic	25	28	Glx	-0.61	3	✓	32.7	76.1	16.2	21.6	38.4	×	7.7
	B	Ohrmann 2008	Chronic	43	37	Glx	0.20	1.5	✓	27.9	65.1	14.1	16.2	34.8	563.6	3.6
	B	Öngür 2008	Chronic	17	21	Glu	-0.55	4	✓	41.8	86.0	22.2	18.1	39.9	542.0	×
						Gln	0.20									
	B	Öngür 2010	Chronic	21	19	Glu	0.45	4	✓	39.0	50.5	-	-	-	555.0	×
	L+R	Purdon 2008	High risk	15	14	Glu	0.48	3	-	46.3	-	-	-	-	-	-
						Glx	0.33									
	L	Rowland 2008	Chronic non deficit	9	6	Glx	-0.47	3	✓	40.0	61.0*	-	-	-	-	19.0
			Chronic deficit	9	6	Glx	0.15	3	✓	43.0	66.0*	-	-	-	-	24.0
	L	Rowland 2013	Chronic young	10	10	Glx	-0.36	3	✓	30.2	63.0*	-	-	-	-	7.7
			Chronic old	10	10	Glx	-0.76	3	✓	51.1	57.0*	-	-	-	-	25.5
	B	Shirayama 2010	Chronic	19	18	Glu	-0.13	3	✓	30.5	40.0*	-	-	-	267.0	7.3
						Gln	0.56									
	B	Stone 2009	High risk	9	12	Gln	1.31	3	-	25.0	-	-	-	-	-	-
	L+R	Tandon 2013	High risk	23	24	Glx	0.50	1.5	-	15.9	-	-	-	-	-	-
	B	Tayoshi 2009	Chronic	30	25	Glu	-0.53	3	✓	33.8	55.2	12.5	15.0	27.7	383.3	10.2
						Gln	-0.34									
	B	Terpstra 2005	Chronic	13	9	Glu	0.23	4	✓	26.0	×	×	×	×	×	×
				12	8	Gln	0.00	4	✓	26.0	×	×	×	×	×	×

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
Medial Frontal Cortex	L	Théberge 2002	FEP	20	20	Glu	-0.14	4	×	26.0	×	×	×	×	-	1.8
						Gln	0.71									
	L	Théberge 2003	Chronic	21	21	Glu	-0.70	4	✓	37.0	×	×	×	×	436.0	15.7
						Gln	-0.70									
	B	Thomas 1998	FEP	12	12	Glx	-0.41	1.5	-	14.0	-	-	-	-	-	-
	R	Tibbo 2004	High risk	20	22	Glx	0.76	3	-	16.4	-	-	-	-	-	-
	B	Tibbo 2013	FEP	33	41	Glu	0.12	3	×	21.6	75.5	18.0	20.0	37.5	-	×
	L, R	Wood 2007	Chronic	15	14	Glx	0.24	3	✓	31.5	58.1	14.2	14.8	29.1	×	9.0
	B	Yoo 2009	High risk	22	22	Glx	0.16	1.5	-	22.6	-	-	-	-	-	-
DLPFC	L	Block 2000	Chronic	25	10	Glx	-0.26	1.5	✓	35.6	-	-	-	-	×	11.5
			High risk	35	10	Glx	-0.50		-	49.2	-	-	-	-	-	-
	L	Da Silva Alves 2011	Chronic	11	23	Glu	-0.04	3	✓	29.3	59.0	10.7	17.6	30.7	×	×
						Gln	0.35									
						Glx	0.22									
	L	Jessen 2013	Chronic	20	20	Gln	0.00	3	✓	34.5	51.0	10.8	13.9	26.4	×	7.2
						Glx	0.16									
	L	Kegeles 2012	Chronic unmedicated	16	11	Glx	0.23	3	×	32.0	71.0	×	×	×	-	7.0
			Chronic medicated	16	11	Glx	0.21	3	✓	32.0	57.0	×	×	×	×	9.0
	-	Ohrmann 2005	FEP	18	11	Glx	-1.80	1.5	×	29.3	85.9	21.8	19.4	43.7	-	2.1
			Chronic	21	11		-0.37		✓	29.7	63.0	12.9	17.1	33.0	×	6.8
	L	Ohrmann 2007	FEP	15	10	Glx	-1.81	1.5	×	27.0	84.6	22.0	19.1	43.6	-	2.3
			Chronic	20	10		-0.33		✓	30.3	61.9	13.1	16.7	32.3	×	7.0
	L	Ohrmann 2008	Chronic	43	37	Glx	0.08	1.5	✓	27.9	65.1	14.1	16.2	34.8	563.6	3.6

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
DLPFC	L	Stanley 1996	FEP naïve	13	8	Glu	0.31	1.5	×	26.0	×	×	×	×	-	2.0
						Gln	-0.37									
			FEP	12	8	Glu	0.64		✓	26.0	×	×	×	×	×	2.5
						Gln	-0.28									
			Chronic	12	8	Glu	-0.61		✓	41.0	×	×	×	×	×	17.0
						Gln	0.76									
	L	Stanley 2007	FEP	18	61	Glu	-0.36	1.5	×	24.0	93.0*	×	×	×	-	×
	L	Tebartz van Elst 2005	Chronic	21	32	Glu	0.72	2	✓	28.5	60.0*	×	×	×	×	5.3
						Gln	0.81									
	L	Yoo 2009	High risk	22	22	Glx	0.31	1.5	-	22.6	-	-	-	-	-	-
Frontal WM	L	Bustillo 2010	FEP	10	10	Glu	-0.12	4	×	27.2	-	-	-	-	-	2.5
						Gln	0.63									
	L, R	Chang 2007	Chronic	23	22	Glx	0.40	4	✓	66.3	75.5	16.5	20.0	39.0	×	43.1
	R	Choe 1994	Chronic	23	10	Glx	0.68	1.5	×	×	-	-	-	-	-	×
	L, R	Choe 1996	Chronic	55	20	Glx	0.66	1.5	×	×	-	-	-	-	-	×
	L	Galińska 2009	FEP	30	19	Glx	-0.52	1.5	✓	22.5	80.2	17.9	22.0	40.3	×	0.8
	L	Lutkenhoff 2010	High risk	12	11	Glu	-0.53	3	-	49.5	-	-	-	-	-	-
			Chronic	9	11	Glu	-0.69	3	✓	48.8	×	×	×	×	×	27.4
	L	Ota 2012	Chronic exacerbated	22	13	Glx	0.08	1.5	✓	45.0	69.2	17.4	17.5	34.3	838.1	20.4
			Chronic not exacerbated	20	13	Glx	0.81	1.5	✓	41.3	56.2	11.7	15.8	28.7	354.2	14.6
	L	Szulc 2004	FEP	31	7	Glx	-0.28	1.5	✓	22.6	79.7	17.6	22.0	39.7	×	×
			Chronic	17	7	Glx	0.03	1.5	✓	33.6	88.6	16.2	24.9	47.5	×	×
	L	Szulc 2011	Chronic	40	25	Glx	0.22	1.5	×	32.2	94.7	18.5	26.1	49.8	300.0	9.2
	L	Tunc-Skarka 2009	FEP + Chronic	26	17	Glu	0.29	3	Both	31.7	×	17.2	16.5	×	207.2	2.0

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
Medial Temporal Lobe	L	Bartha 1999	FEP	11	11	Glu	-0.22	1.5	×	27.4	-	-	-	-	-	×
						Gln	0.12									
	L	Bloemen 2011	High risk	11	11	Glu	-0.98	3	-	21.3	-	-	-	-	-	-
	L	Capizzano 2011	HR (1st degree relative)	15	12	Glx	-0.42	3	-	19.3	-	-	-	-	-	-
			HR (2nd degree relative)	20	12	Glx	0.22	3	-	19.5	-	-	-	-	-	-
	L, R	Chang 2007	Chronic	23	22	Glx	0.92	4	✓	66.3	75.5	16.5	20.0	39.0	×	43.1
	L	Da Silva Alves 2011	Chronic	9	16	Glu	0.87	3	✓	29.3	59.0	10.7	17.6	30.7	×	×
						Gln	0.69									
						Glx	1.23									
	L	Galińska 2009	FEP	30	19	Glx	0.14	1.5	✓	22.5	80.2	17.9	22.0	40.3	×	0.8
	L	Hasan 2014	FEP	46	49	Glx	-0.11	1.5	✓	29.2	91.9	21.7	22.4	47.8	290.0	
	L	Hutcheson 2012	Chronic	28	28	Glx	0.00	3	✓	36.7	72.0*	×	×	×	×	15.9
	L, R	Kegeles 2000	Chronic	10	10	Glx	0.17	1.5	×	28.0	59.0*	×	×	×	-	8.0
	L	Kraguljac 2012	Chronic	48	46	Glx	-0.10	3	✓	38.0	58.0*	×	×	×	×	16.4
	L	Kraguljac 2013	Chronic	27	27	Glx	0.61	3	×	32.6	79.0*	×	×	×	-	10.5
	L	Lutkenhoff 2010	High risk	12	11	Glu	0.03	3	-	49.5	-	-	-	-	-	-
			Chronic	9	11	Glu	-0.38	3	✓	48.8	×	×	×	×	×	27.4
	L	Stan 2014	Chronic	18	16	Glu	-0.69	3	Both	41.9	×	×	×	×	×	×
	L	Stone 2009	High risk	24	27	Glu	-0.23	3	-	25.0	-	-	-	-	-	-
				5	4	Gln	0.55									
				24	27	Glx	0.13									
	L	Szulc 2004	FEP	31	7	Glx	0.82	1.5	✓	22.6	79.7	17.6	22.0	39.7	×	×
			Chronic	17	7	Glx	0.43	1.5	✓	33.6	88.6	16.2	24.9	47.5	×	×
	L	Szulc 2011	Chronic	36	25	Glx	0.37	1.5	×	32.2	94.7	18.5	26.1	49.8	300.0	9.2

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
Medial Temporal Lobe	L	Tebartz van Elst 2005	Chronic	21	32	Glu	0.65	2	✓	28.5	60.0*	×	×	×	×	5.3
						Gln	0.39									
	L, R	Wood 2008	FEP medication naïve	12	10	Glx	0.16	4	×	20.2	74.4	20.8	14.3	40.0	-	×
			FEP early treated	13	10	Glx	0.38	4	✓	20.3	90.3	21.9	18.7	47.0	×	×
	L, R	Wood 2010	High risk transition	6	15	Glx	1.52	3	-	×	-	-	-	-	-	-
			High risk no transition	55	15	Glx	0.63	3	-	×	-	-	-	-	-	-
Thalamus	L	Bustillo 2010	FEP	12	10	Glu	0.20	4	×	27.2	-	-	-	-	-	2.5
						Gln	-0.08									
	L	Egerton 2014	High risk	75	55	Glu	-0.41	3	-	23.3	-	-	-	-	-	-
						Glx	-0.23									
	L	Galińska 2009	FEP	30	19	Glx	0.14	1.5	✓	22.5	80.2	17.9	22.0	40.3	×	0.8
	L	Szulc 2004	FEP	31	7	Glx	0.32	1.5	✓	22.6	79.7	17.6	22.0	39.7	×	×
			Chronic	17	7	Glx	-0.03	1.5	✓	33.6	88.6	16.2	24.9	47.5	×	×
	L	Szulc 2011	Chronic	42	26	Glx	-0.09	1.5	×	32.2	94.7	18.5	26.1	49.8	300.0	9.2
	L+R	Tandon 2013	High risk	23	24	Glx	0.69	1.5	-	15.9	-	-	-	-	-	-
	L	Théberge 2002	FEP	19	19	Glu	0.03	4	×	26.0	×	×	×	×	-	1.8
						Gln	0.70									
	L	Théberge 2003	Chronic	19	19	Glu	-0.01	4	✓	37.0	×	×	×	×	436.0	15.7
						Gln	0.89									
	L	Yoo 2009	High risk	22	22	Glx	0.12	1.5	-	22.6	-	-	-	-	-	-

Brain Region and Hemisphere		Study and Year	Patient group	Cases *n*	Control *n*	Metabolite	Effect size	Field strength	Currently Medicated	Age	PANSS subscale				CPZ	Duration illness
											Total	Positive	Negative	General		
Basal Ganglia	L	Block 2000	Chronic	25	10	Glx	0.31	1.5	✓	35.6	-	-	-	-	×	11.5
			High risk	35	10	Glx	0.06	1.5	-	49.2	-	-	-	-	-	-
	R	de la Fuente-Sandoval 2011	High risk	18	20	Glu	0.86	3	-	19.6	-	-	-	-	-	-
						Glx	0.40									
			FEP	18	20	Glu	0.97	3	×	23.4	95.1	22.2	25.7	47.1	-	×
						Glx	0.66									
	R	de la Fuente-Sandoval 2013	FEP	23	18	Glu	0.80	3	×	26.6	94.5	23.3	24.1	47.9	-	×
						Glx	0.63									
	L	Goto 2012	FEP	18	18	Glx	0.69	3	✓	31.0	68.1	15.9	17.2	34.2	293.4	0.6
	L+R	Keshavan 2009	High risk	40	48	Glx	-0.34	1.5	-	×	-	-	-	-	-	-
	L+R	Tandon 2013	High risk	23	24	Glx	0.88	1.5	-	15.9	-	-	-	-	-	-
	L	Tayoshi 2009	Chronic	30	25	Glu	0.00	3	✓	33.8	55.2	12.5	15.0	27.7	383.3	10.2
						Gln	0.00									
	L	Yamasue 2003	Chronic	16	15	Glx	0.49	1.5	✓	30.7	68.5	14.5	19.5	34.5	543.0	8.0
Cerebellum	R	de la Fuente-Sandoval 2011	High risk	18	20	Glu	-0.17	3	-	19.6	-	-	-	-	-	-
						Glx	-0.07									
			FEP	18	20	Glu	0.69	3	×	23.4	95.1	22.2	25.7	47.1	-	×
						Glx	0.70									
	R	de la Fuente-Sandoval 2013	FEP	23	18	Glu	0.63	3	×	26.6	94.5	23.3	24.1	47.9	-	×
						Glx	0.05									

eTable 2. Meta-analysis Results Summary for Patients With Schizophrenia (First Episode Psychosis and Chronic Schizophrenia) in All Brain Regions

Brain region	Metabolite	Number of Studies	Studies	Cases	Healthy Volunteers	Effect size (95% CI)		Effect size P value	Heterogeneity I^2 %	Heterogeneity P value
Medial frontal cortex	Glutamate	3 FEP, 11 SZ	14	309	302	-0.16	(-0.38 to 0.07)	0.169	38.4	0.071
	Glutamine	2 FEP, 3 SZ	5	84	78	0.18	(-0.38 to 0.76)	0.519	67.7	0.015
	Glx	3 FEP, 13 SZ	16	284	257	0.02	(-0.17 to 0.21)	0.861	12.2	0.314
DLPFC	Glx	2 FEP, 8 SZ	10	205	153	-0.32	(-0.75 to 0.11)	0.142	71.6	<0.001
Frontal WM	Glutamate	1 FEP, 1 C, 1 FEP+SZ	3	45	37.5	-0.09	(-0.65 to 0.47)	0.755	33.7	0.221
	Glx	2 FEP, 7 SZ	9	261	135	0.24	(-0.06 to 0.54)	0.113	46.2	0.062
MTL	Glutamate	0 FEP, 3 SZ	3	36	42.5	-0.08	(-1.02 to 0.86)	0.867	75.0	0.018
	Glx	**5 FEP, 8 SZ**	**13**	**329**	**272**	**0.31**	**(0.09 to 0.53)**	**0.007***	**39.7**	**0.069**
Basal Ganglia	Glutamate	2 FEP, 1 SZ	3	71	63	0.56	(-0.06 to 1.18)	0.076	67.4	0.047
	Glx	**3 FEP, 2 SZ**	**5**	**100**	**81**	**0.57**	**(0.26 to 0.88)**	**<0.001***	**0.0**	**0.950**
Thalamus	Glutamate	2 FEP, 1 SZ	3	50	48	0.05	(-0.34 to 0.45)	0.791	0.0	0.924
	Glutamine	**2 FEP, 1 SZ**	**3**	**50**	**48**	**0.56**	**(0.02 to 1.10)**	**0.042**	**40.5**	**0.186**
	Glx	2 FEP, 2 SZ	4	120	58	0.05	(-0.27 to 0.37)	0.776	0.0	0.840

DLPFC; Dorsolateral prefrontal cortex, Frontal WM; Frontal white matter, MTL; Medial temporal lobe. FEP; first episode psychosis, SZ; chronic schizophrenia patients. * indicate results which survive multiple comparisons for each region as glutamate, Glx and glutamine were investigated.

eTable 3. List of Studies Included in the Meta-analysis and Methodological Variables

FEP; first episode psychosis. Medial frontal; medial prefrontal cortex + anterior cingulate cortex, DLPFC; dorsolateral prefrontal cortex, frontal WM; frontal white matter. L; Left, R; Right, B; Bilateral. Glu; glutamate, Gln; glutamine. FWHM; full width at half maximum, CRLB; Cramér–Rao Lower Bound, CSF-correction; refers to the correction for the presence of cerebrospinal fluid. STEAM; STimulated Echo Acquisition Mode, PRESS; Point resolved spectroscopy, MEGA-PRESS; MEshcher–GArwood Point RESolved Spectroscopy, sLASER; semi-localized by adiabatic selective refocusing. Ppm; parts per million, (ms); miliseconds.

Brain Region and Hemisphere		Study and Year	Patient group	Cases n	Control n	Metabolite	Field strength	Acquisition sequence	Echo time (ms)	FWHM	CRLB threshold	Creatine scaling / CSF-correction
Medial frontal	L	Bartha 1997	FEP	10	10	Glu, Gln	1.5	STEAM	20	×	×	×
	B	Bustillo 2010	FEP	14	8	Glu, Gln	4	STEAM	20	<13 Hz	20%	CSF-correction
	B	Bustillo 2013	Chronic	84	81	Glu	3	PRESS	40	×	20%	CSF-correction
				72	76	Gln					30%	
	B	Capizzano 2011	HR (1st degree relative)	12	10	Glx	3	PRESS	30	<0.1 ppm	20%	Cr-scaling
	B		HR (2nd degree relative)	12	10	Glx	3					
	B	Demjaha 2013	Chronic responder	8	5	Glu, Glx	3	PRESS	30	×	20%	CSF-correction
			Chronic treatment resistant	6	5	Glu, Glx	3					
	B	Egerton 2014	High risk	75	55	Glu, Glx	3	PRESS	30	×	20%	CSF-correction
	B	Goto 2012	FEP	18	18	Glx	3	MEGA-PRESS	68	×	×	Cr-scaling
	L	Jessen 2013	Chronic	20	20	Gln, Glx	3	PRESS	30	<6 Hz	×	Cr-scaling
	B	Kegeles 2012	Chronic unmedicated	16	11	Glx	3	PRESS	68	×	×	Cr-scaling
			Chronic medicated	16	11	Glx	3					
	B	Kraguljac 2012	Chronic	48	46	Glx	3	PRESS	80	<25 Hz	30%	Cr-scaling
	B	Lutkenhoff 2010	High risk	12	11	Glu	3	PRESS	30	<0.1 ppm	25%	CSF-correction
			Chronic	9	11	Glu	3					
	B	Marsman 2014	Chronic	14	18	Glu	7	sLASER	28	9 Hz	20%	CSF-correction
	B	Natsubori 2014	High risk	24	26	Glx	3	STEAM	15	0.16 ppm	20%	CSF-correction
			FEP	19	19	Glx	3					
			Chronic	25	28	Glx	3					

Brain Region and Hemisphere		Study and Year	Patient group	Cases n	Control n	Metabolite	Field strength	Acquisition sequence	Echo time (ms)	FWHM	CRLB threshold	Creatine scaling / CSF-correction
Medial frontal	B	Ohrmann 2008	Chronic	43	37	Glx	1.5	PRESS	32	×	20%	×
	B	Öngür 2008	Chronic	17	21	Glu, Gln	4	PRESS	30 to 500	9 Hz	×	×
	B	Öngür 2010	Chronic	21	19	Glu	4	MEGA-PRESS	68	×	×	Cr-scaling
	L+R	Purdon 2008	High risk	15	14	Glu, Glx	3	STEAM	20	×	25%	Cr-scaling
	L	Rowland 2008	Chronic non deficit Chronic deficit	9 9	6 6	Glx Glx	3 3	PRESS	35	<0.1 ppm	20%	×
	L	Rowland 2013	Chronic young Chronic old	10 10	10 10	Glx Glx	3 3	PRESS	35	<0.1 ppm	20%	CSF-correction
	B	Shirayama 2010	Chronic	19	18	Glu, Gln	3	PRESS	30	×	25%	×
	B	Stone 2009	High risk	9	12	Gln	3	PRESS	30	×	20%	CSF-correction
	L+R	Tandon 2013	High risk	23	24	Glx	1.5	PRESS	30	×	20%	CSF-correction
	B	Tayoshi 2009	Chronic	30	25	Glu, Gln	3	STEAM	18	×	×	CSF-correction
	B	Terpstra 2005	Chronic	13 12	9 8	Glu Gln	4 4	STEAM	5	6–11 Hz	20%	×
	L	Théberge 2002	FEP	20	20	Glu, Gln	4	STEAM	20	×	×	×
	L	Théberge 2003	Chronic	21	21	Glu, Gln	4	STEAM	20	×	×	×
	B	Thomas 1998	FEP	12	12	Glx	1.5	STEAM	20	×	×	Cr-scaling
	R	Tibbo 2004	High risk	20	22	Glx	3	STEAM	20	×	×	Cr-scaling
	B	Tibbo 2013	FEP	33	41	Glu	3	STEAM	240	0.05 ppm	×	×
	L, R	Wood 2007	Chronic	15	14	Glx	3	PRESS	30	0.07 ppm	×	×
	B	Yoo 2009	High risk	22	22	Glx	1.5	PRESS	140	×	25%	CSF-correction

Brain Region and Hemisphere		Study and Year	Patient group	Cases n	Control n	Metabolite	Field strength	Acquisition sequence	Echo time (ms)	FWHM	CRLB threshold	Creatine scaling / CSF-correction
DLPFC	L	Block 2000	Chronic	25	10	Glx	1.5	PRESS	30	×	×	Creatine scaling
			High risk	35	10	Glx						
	L	Da Silva Alves 2011	Chronic	11	23	Glu, Gln, Glx	3	PRESS	36	<0.1 ppm	15%, 50%, 15%	×
	L	Jessen 2013	Chronic	20	20	Gln, Glx	3	PRESS	30	<6 Hz	×	Cr-scaling
	L	Kegeles 2012	Chronic unmedicated	16	11	Glx	3	PRESS	68	×	×	Cr-scaling
			Chronic medicated	16	11	Glx	3					
	-	Ohrmann 2005	FEP	18	11	Glx	1.5	-	-	×	20%	CSF-correction
			Chronic	21	11							
	L	Ohrmann 2007	FEP	15	10	Glx	1.5	STEAM	20	×	20%	CSF-correction
			Chronic	20	10							
	L	Ohrmann 2008	Chronic	43	37	Glx	1.5	PRESS	32	×	20%	×
	L	Stanley 1996	FEP naïve	13	8	Glu, Gln	1.5	STEAM	20	×	×	×
			FEP	12	8							
			Chronic	12	8							
	L	Stanley 2007	FEP	18	61	Glu	1.5	STEAM	20	×	35%	CSF-correction
	L	Tebartz van Elst 2005	Chronic	21	32	Glu, Gln	2	PRESS	30	×	×	CSF-correction
	L	Yoo 2009	High risk	22	22	Glx	1.5	PRESS	140	×	25%	CSF-correction

Brain Region and Hemisphere		Study and Year	Patient group	Cases n	Control n	Metabolite	Field strength	Acquisition sequence	Echo time (ms)	FWHM	CRLB threshold	Creatine scaling / CSF-correction
Frontal WM	L	Bustillo 2010	FEP	10	10	Glu, Gln	4	STEAM	20	<13 Hz	30%	CSF-correction
	L, R	Chang 2007	Chronic	23	22	Glx	4	PRESS	30	<0.1 ppm	20%	×
	R	Choe 1994	Chronic	23	10	Glx	1.5	PRESS	30	×	×	Creatine scaling
	L, R	Choe 1996	Chronic	55	20	Glx	1.5	STEAM	20	×	×	Creatine scaling
	L	Galińska 2009	FEP	30	19	Glx	1.5	PRESS	35	5-7 Hz	×	Creatine scaling
	L	Lutkenhoff 2010	High risk	12	11	Glu	3	PRESS	30	<0.1 ppm	25%	CSF-correction
			Chronic	9	11	Glu	3					
	L	Ota 2012	Chronic exacerbated	22	13	Glx	1.5	PRESS	30	×	20%	×
			Chronic not exacerbated	20	13	Glx	1.5					
	L	Szulc 2004	FEP	31	7	Glx	1.5	PRESS	35	×	×	Creatine scaling
			Chronic	17	7	Glx	1.5					
	L	Szulc 2011	Chronic	40	25	Glx	1.5	PRESS	35	3-7 Hz	×	Creatine scaling
	L	Tunc-Skarka 2009	FEP + Chronic	26	17	Glu	3	PRESS	30	×	20%	CSF-correction

Brain Region and Hemisphere		Study and Year	Patient group	Cases n	Control n	Metabolite	Field strength	Acquisition sequence	Echo time (ms)	FWHM	CRLB threshold	Creatine scaling / CSF-correction
Medial Temporal Lobe	L	Bartha 1999	FEP	11	11	Glu, Gln	1.5	STEAM	20	6.2 Hz	×	×
	L	Bloemen 2011	High risk	11	11	Glu	3	PRESS	36	×	×	×
	L	Capizzano 2011	HR (1st degree relative)	15	12	Glx	3	PRESS	30	<0.1 ppm	20%	Cr-scaling
			HR (2nd degree relative)	20	12	Glx	3					
	L, R	Chang 2007	Chronic	23	22	Glx	4	PRESS	30	<0.1 ppm	20%	×
	L	Da Silva Alves 2011	Chronic	9	16	Glu, Gln, Glx	3	PRESS	36	<0.1 ppm	15%, 50%, 15%	×
	L	Galińska 2009	FEP	30	19	Glx	1.5	PRESS	35	5-7 Hz	×	Creatine scaling
	L	Hasan 2014	FEP	46	49	Glx	1.5	-	30	×	×	Creatine scaling
	L	Hutcheson 2012	Chronic	28	28	Glx	3	PRESS	80	<25 Hz	30%	Creatine scaling
	L, R	Kegeles 2000	Chronic	10	10	Glx	1.5	STEAM	20	0.1-0.25 ppm	×	Creatine scaling
	L	Kraguljac 2012	Chronic	48	46	Glx	3	PRESS	80	<25 Hz	30%	Cr-scaling
	L	Kraguljac 2013	Chronic	27	27	Glx	3	PRESS	80	<25 Hz	25%	Cr-scaling
	L	Lutkenhoff 2010	High risk	12	11	Glu	3	PRESS	30	<0.1 ppm	25%	CSF-correction
			Chronic	9	11	Glu	3					
	L	Stan 2014	Chronic	18	16	Glu	3	PRESS	70	×	×	Creatine scaling
	L	Stone 2009	High risk	24	27	Glu, Glx	3	PRESS	30	×	20%	CSF-correction
				5	4	Gln						
	L	Szulc 2004	FEP	31	7	Glx	1.5	PRESS	35	×	×	Creatine scaling
			Chronic	17	7	Glx	1.5					
	L	Szulc 2011	Chronic	36	25	Glx	1.5	PRESS	35	3-7 Hz	×	Creatine scaling
	L	Tebartz van Elst 2005	Chronic	21	32	Glu, Gln	2	PRESS	30	×	×	CSF-correction
	L, R	Wood 2008	FEP medication naïve	12	10	Glx	4	PRESS	30	0.09 ppm	35%	×
			FEP early treated	13	10	Glx	4					
	L, R	Wood 2010	HR transition	6	15	Glx	3	PRESS	30	12.4 ppm	30%	×
			HR no transition	55	15	Glx	3					

Brain Region and Hemisphere		Study and Year	Patient group	Cases n	Control n	Metabolite	Field strength	Acquisition sequence	Echo time (ms)	FWHM	CRLB threshold	Creatine scaling / CSF-correction
Thalamus	L	Bustillo 2010	FEP	12	10	Glu, Gln	4	STEAM	20	<13 Hz	30%	CSF-correction
	L	Egerton 2014	High risk	75	55	Glu, Glx	3	PRESS	30	×	20%	CSF-correction
	L	Galińska 2009	FEP	30	19	Glx	1.5	PRESS	35	5-7 Hz	×	Creatine scaling
	L	Szulc 2004	FEP	31	7	Glx	1.5	PRESS	35	×	×	Creatine scaling
			Chronic	17	7	Glx	1.5					
	L	Szulc 2011	Chronic	42	26	Glx	1.5	PRESS	35	3-7 Hz	×	Creatine scaling
	L+R	Tandon 2013	High risk	23	24	Glx	1.5	PRESS	30	×	20%	CSF-correction
	L	Théberge 2002	FEP	19	19	Glu, Gln	4	STEAM	20	×	×	×
	L	Théberge 2003	Chronic	19	19	Glu, Gln	4	STEAM	20	×	×	×
	L	Yoo 2009	High risk	22	22	Glx	1.5	PRESS	140	×	25%	CSF-correction
Basal Ganglia	L	Block 2000	Chronic	25	10	Glx	1.5	PRESS	30	×	×	Creatine scaling
			High risk	35	10	Glx	1.5					
	R	de la Fuente-Sandoval 2011	High risk	18	20	Glu, Glx	3	PRESS	35	<12 Hz	20%	CSF-correction
			FEP	18	20	Glu, Glx	3					
	R	de la Fuente-Sandoval 2013	FEP	23	18	Glu, Glx	3	PRESS	35	<12 Hz	20%	CSF-correction
	L	Goto 2012	FEP	18	18	Glx	3	MEGA-PRESS	68	×	×	Cr-scaling
	L+R	Keshavan 2009	High risk	40	48	Glx	1.5	PRESS	30	×	23%	CSF-correction
	L+R	Tandon 2013	High risk	23	24	Glx	1.5	PRESS	30	×	20%	CSF-correction
	L	Tayoshi 2009	Chronic	30	25	Glu, Gln	3	STEAM	18	×	×	CSF-correction
	L	Yamasue 2003	Chronic	16	15	Glx	1.5	PRESS	35	<6 Hz	×	×
Cerebellum	R	de la Fuente-Sandoval 2011	High risk	18	20	Glu, Glx	3	PRESS	35	<12 Hz	20%	CSF-correction
			FEP	18	20	Glu, Glx	3					
	R	de la Fuente-Sandoval 2013	FEP	23	18	Glu, Glx	3	PRESS	35	<12 Hz	20%	CSF-correction

eResults. Patient Details

Fourteen studies examined participants at high risk for psychosis. Of these, six examined high risk subjects identified on the basis of sub-clinical symptoms[1–6], and eight examined participants at familial high risk, including the offspring, siblings or co-twins of patients with schizophrenia[7–11], first and second degree relatives of patients with schizophrenia[12], subjects with at least two relatives with schizophrenia[13], and unspecified relatives of patients with schizophrenia[14].

Eighteen studies examined patients experiencing a first episode of psychosis (FEP), all with an onset of illness within the last 2 and a half years. Of these, eleven samples comprised patients who were naïve to antipsychotic medication[15–17,1,18–24], with the exception of one patient in one study[20], who received two tablets of haloperidol 2 years before the study began. Two studies included FEP samples who were minimally-treated; in one most patients were antipsychotic naïve, but a minority had received risperidone 24 hours before the study[25] and in the other, patients had a lifetime antipsychotic medication exposure of less than three weeks, and had received antipsychotic medication for an average of 5±6 days prior to scanning[26]. Seven studies examined patients still within their first psychotic episode but who had received antipsychotic medication[27,18,28–30,22,5].

Thirty-six studies examined patients with established (or 'chronic') schizophrenia. Most (twenty-eight) of these included patients who had been treated with antipsychotic medication[31–35,18,28,36,14,37–46,11,47,48,5,23,24,49,50]. One study examined patients with 22q11 deletion and schizophrenia[51]. Four studies examined patients with schizophrenia who were not taking medication at the time of scanning[52–55]. One study compared antipsychotic-treated versus antipsychotic-free patients[56] and another included both antipsychotic treated and antipsychotic-free participants[57]. One study examined patients with schizophrenia who were medication naïve[58]. Finally, one study investigated a mixture of unmedicated first episode patients and medicated chronic patients, and so was included in analyses of all patients but not in analyses separating clinical groups[59].

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Funnel plots

Funnel plots are presented for regions in which significant glutamatergic differences were found between cases and controls in the meta-analysis. The effect size estimates from individual studies are plotted against their standard error. Standard error is plotted on an inverse scale, so more powerful studies appear at the top of graph. As the variance of the effect estimate is not the same for all studies, each estimate has been divided by its standard error.

Funnel plots can be useful in assessing publication bias, as studies with larger variance will scatter along the bottom of the graph, whereas more precise studies will aggregate towards the true effect size. Asymmetric funnel plots may indicate publication bias, as smaller studies that did not find significant differences are not published.

A funnel plot asymmetry test is recommended when there are at least 10 studies included in the meta-analysis, and so funnel plots are not presented for glutamate in the basal ganglia (4 studies) and glutamine in the thalamus (3 studies). As publication bias does not necessarily cause asymmetry in funnel plots, Egger's regression test has also been performed (see Chapter 2.2) which is a more quantitative method of assessing publication bias. Small study bias, which may be evident of publication bias, was evident for reports of Glx in the medial temporal lobe (Egger test; *P*=0.025) and Glx in the basal ganglia (*P*=0.04).

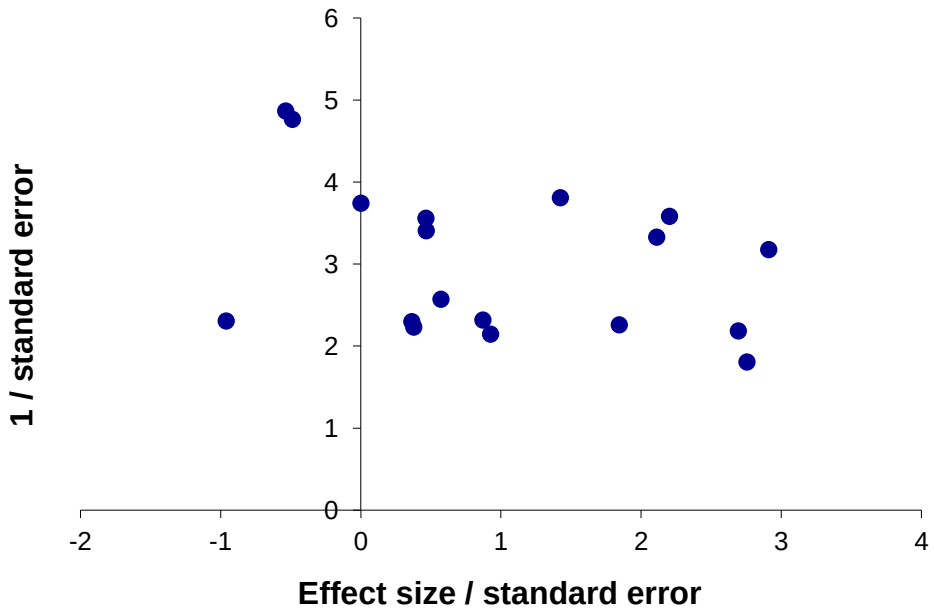

Figure 7 Funnel plot of studies standardised effect sizes for Glx differences between cases and controls in the Medial Temporal Lobe. Egger test; *P*=0.025

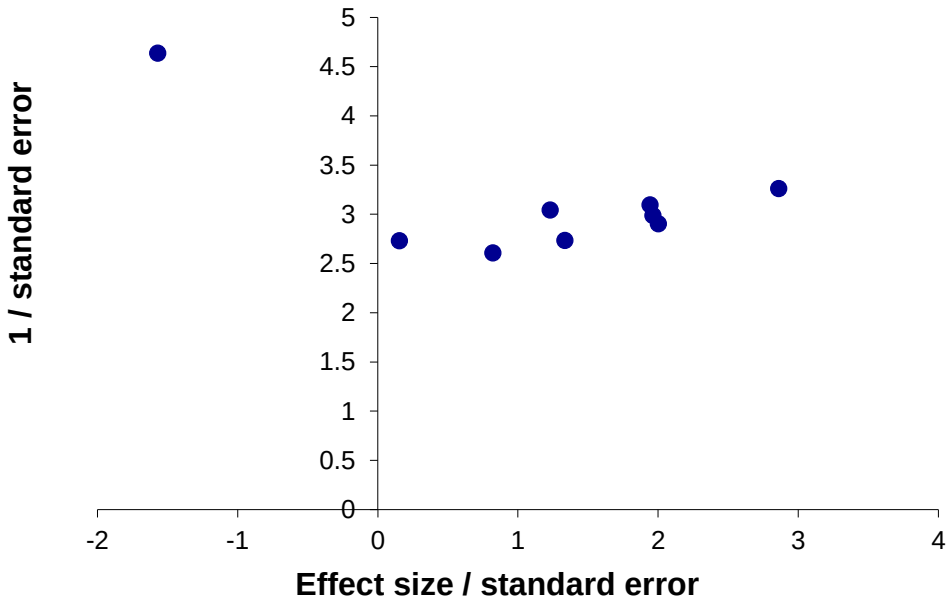

Figure 8 Funnel plot of studies standardised effect sizes for Glx differences between cases and controls in the Basal Ganglia. Egger test; *P*=0.04

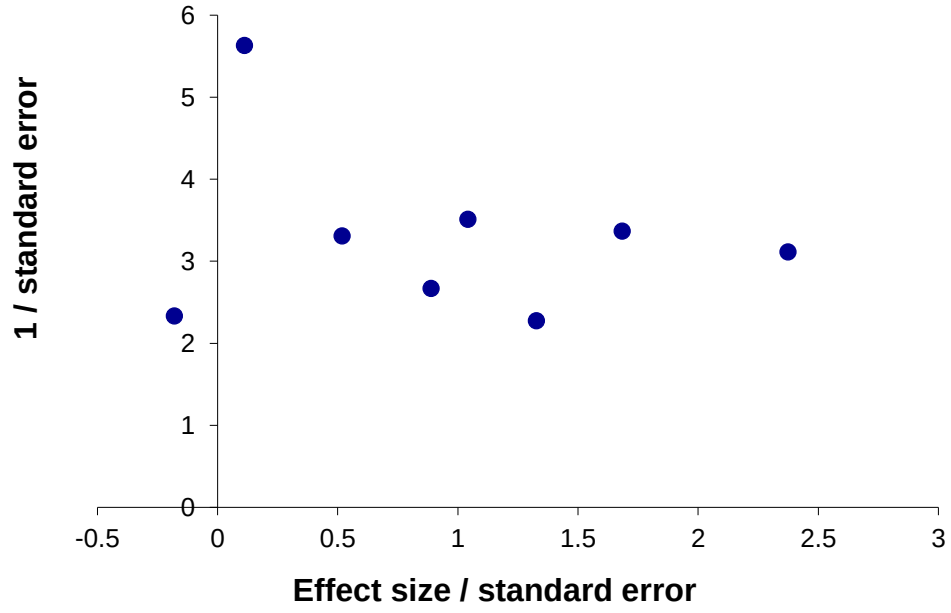

Figure 9 Funnel plot of studies standardised effect sizes for Glx differences between high risk subjects and controls in the Medial Frontal Cortex. Egger test; *P*=0.20

2.3. A Multicentre analysis of proton magnetic resonance spectroscopy studies and their relation to symptoms

The aim of this study was to investigate the relation of 1H-MRS glutamate measures to symptom severity and medication status in a large dataset of individual patient data collected at multiple centres. While the meta-regression (section 2.2) did not find evidence of relationships with symptom severity or medication, analysis of individual patient data will have greater power to detect these effects as meta-regression relies on group mean values. I hypothesised that more symptomatic patients would possess higher glutamatergic metabolite levels, based on findings from previous studies comparing patients on the basis of symptom severity (Egerton et al., 2012; Ota et al., 2012). I also predicted that the dose of antipsychotic medication would directly correlate with frontal and striatal glutamate metabolite concentrations, on the basis of recent findings of lower medial prefrontal Glx in unmedicated patients relative to both medicated patients and healthy controls (Kegeles et al., 2012) and reduced striatal glutamate levels in FEP patients following treatment (de la Fuente-Sandoval et al., 2013). Consistent with the aims of the meta-analysis (section 2.2), this study aimed to investigate differences in glutamate levels between patients and controls, and whether these differences vary according to illness stage. Based on the meta-analysis findings we expect that patients with schizophrenia are associated with elevations in glutamatergic metabolites, and that these vary with the stage of disorder.

2.3.1. Methods

The authors of the published studies included in the meta-analysis (see section 2.2) were contacted and asked to provide individual glutamate and Glx values for schizophrenia patients and controls, as well as individual creatine (Cr) values to allow Cr-scaling of glutamatergic metabolites. PANSS positive, negative and general subscores, CPZ equivalent doses and medication status for each subject were also requested.

Studies which did not report healthy control comparisons, and had therefore not been included in the main meta-analysis (section 2.2), were also contacted (Egerton et al., 2012; Goff et al., 2002; Szulc et al., 2005), as healthy volunteer data were not necessary for analyses of symptom severity and medication exposure within a patient group. The radiofrequency-pulse edited metabolite values reported in (Kegeles et al., 2012) could not be included as these are not comparable to non-edited spectra values obtained in the majority of studies. The primary outcome measures were CSF-corrected glutamate, glutamine and Glx values. However Cr-scaled glutamatergic metabolites were assessed

instead if a greater number of studies provided Cr-scaled than CSF-corrected values for a brain region.

Statistical analysis was performed in SPSS version 22.0 (SPSS, Chicago, IL, USA). Univariate ANOVA determined whether glutamatergic metabolite values significantly differed between sites. As some sites used more than one scanner, site was defined according to the specific MRI scanner used at a geographical site. Differences in glutamatergic metabolite levels between cases and healthy controls were assessed using linear mixed-effects models controlling for site as a random factor. To determine whether glutamatergic abnormalities were specific to a clinical stage of disease, secondary analyses assessed high risk subjects, FEP and chronic schizophrenia patients separately in comparison to their corresponding controls.

Linear mixed-effects models were used to assess the relationships between glutamatergic measures in schizophrenia patients and continuous outcomes (symptom scores and CPZ equivalent doses) or categorical outcomes (presence or absence of medication), with site entered as a random factor. Correlations between glutamatergic metabolites and symptom scores (PANSS positive, negative and general subscales) were corrected for multiple comparisons, giving an adjusted threshold of P=0.017. High risk subjects were excluded from symptom score, CPZ equivalent dose and medication analyses, as this group generally have lower psychotic symptom severities than patients with schizophrenia, and are not usually treated with antipsychotic medication.

2.3.2. Results

Table 1 lists the studies and measures acquired from multiple sites. Individual data were contributed by 17 studies (de la Fuente-Sandoval et al., 2013, 2011; Demjaha et al., 2014; Egerton et al., 2014, 2012; Galińska et al., 2009; Goto et al., 2012; Lawrence S Kegeles et al., 2000; Natsubori et al., 2014; Stone et al., 2010; Szulc et al., 2011; Theberge et al., 2002; Théberge et al., 2003; Tibbo et al., 2013; Wood et al., 2008, 2007; Yamasue et al., 2003), which reflected 10 sites, and values in 531 cases and 404 controls.

Studies identified as using the same scanner at a particular site were: Galinska (2009) and Szulc (2011); Wood (2007) and Wood (2008); Theberge (2002) and Theberge (2003); Fuente-Sandoval (2011) and Fuente-Sandoval (2013); and finally Stone (2009), Egerton (2012), Egerton (2014), and Demjaha (2013). Yamasue (2003) and Natsubori (2013) used different scanners at the same site and so were coded separately. Glutamatergic values were available for more than 3 studies for the following brain regions: medial frontal

cortex, thalamus, striatum and medial temporal lobe. Univariate ANOVA analyses revealed significant site differences in glutamate and Glx values for all brain regions. Therefore, site was included as a random factor in the linear mixed-effects models.

Table 1 Multicentre data for 1H-MRS glutamate measures in a number of brain regions.

Linear mixed-effects model analysis assessed the difference in glutamate measures in cases, high risk subjects, FEP and schizophrenia patients relative to controls. Glutamate metabolite differences between medicated and unmedicated patients were also assessed. Mean and standard errors (SE) adjusted for site are shown. "-" indicates values were only available for one study.

Medial Frontal Cortex										
	# Studies	# Sites	Cases			Controls			Sig	
			n	Mean	SE	*n*	Mean	SE	F	*P*
Glutamate (CSF-corrected)										
All cases	5	3	206	12.01	2.16	179	12.22	2.16	0.54	0.46
High risk	2	2	101	10.76	2.59	82	10.69	2.60	0.03	0.87
FEP	3	3	48	12.70	2.35	96	12.58	2.34	0.08	0.78
Chronic	3	3	57	10.20	2.11	59	11.12	2.11	4.15	**0.04**
Medicated	3	2	40	12.29	1.41				8.01	**0.01**
Medication-free	2	2	24	14.73	1.50					
Glx (CSF-corrected)										
All cases	3	2	170	15.05	2.67	140	14.84	2.67	0.17	0.68
High risk	2	2	103	15.72	2.80	83	15.27	2.81	0.40	0.53
FEP	2	2	28	15.95	3.16	77	15.17	3.11	0.73	0.39
Chronic	2	2	39	12.00	0.67	39	12.69	0.68	1.31	0.26
Medicated	3	2	62	13.49	2.03					-
Medication-free	1	1	5	16.60	2.64					
Glutamine (CSF-corrected)										
All cases	5	2	79	7.94	0.30	63	7.74	0.34	0.20	0.66
High risk	1	1	25	8.78	0.64	19	7.37	0.74		-
FEP	2	2	27	8.34	0.40	39	7.40	0.33	3.32	0.07
Chronic	2	2	27	6.27	1.07	24	7.65	1.10	4.34	**0.04**
Medicated	4	2	32	7.00	0.39				4.72	**0.03**
Medication-free	3	2	22	8.34	0.48					

Thalamus										
	# Studies	# Sites	Cases			Controls			Sig	
			n	Mean	SE	*n*	Mean	SE	F	*P*
Glutamate (CSF corrected)										
All cases	4	2	136	10.25	3.19	105	10.48	3.19	0.93	0.34
High risk	1	1	80	7.01	0.97	57	7.65	0.97		-
FEP	2	2	26	10.80	3.22	76	10.82	3.20	0.00	0.97
Chronic	2	2	30	9.85	3.43	29	9.52	3.43	0.60	0.44
Medicated	3	2	35	9.99	3.11				1.99	0.17
Medication-free	2	2	21	10.98	3.14					
Glx (CSF corrected)										
All cases	2	1	97	8.64	0.23	65	9.03	0.28	1.17	0.28
High risk	1	1	76	8.74	0.26	57	9.35	0.30		-
FEP	1	1	9	8.47	0.84	57	9.35	0.33		-
Chronic	1	1	12	8.09	0.58	8	6.73	0.71		-
Medicated	2	1	17	8.18	1.32					-
Medication-free	1	1	4	8.54	1.64					

Striatum										
	# Studies	# Sites	Cases			Controls			Sig	
			n	Mean	SE	*n*	Mean	SE	F	*P*
Glutamate (CSF corrected)										
All cases	2	1	60	29.21	0.59	58	25.38	0.60	20.53	**<0.001**
High risk	1	1	18	27.54	2.71	40	24.09	2.63		-
FEP	2	1	42	29.93	0.70	58	25.38	0.60	24.62	**<0.001**
Chronic	0	0	0	-	-					
Medicated	0	0	0	-	-					-
Medication-free	2	1	42	29.93	4.00					
Glx (Cr scaled)										
All cases	4	3	94	1.33	0.35	91	1.27	0.35	4.52	**0.04**
High risk	1	1	18	1.88	0.04	40	1.88	0.03		-
FEP	3	2	60	1.30	0.61	76	1.24	0.61	2.82	0.10
Chronic	1	1	16	1.42	0.14	15	1.30	0.14		**-**
Medicated	2	2	34	1.07	0.34				1.90	0.40*
Medication-free	2	1	42	1.89	0.49					

*medication status is not independent from site.

Medial temporal Lobe										
	# Studies	# Sites	Cases			Controls			Sig	
			n	Mean	SE	*n*	Mean	SE	F	*P*
Glx (Cr scaled)										
All cases	5	4	129	1.60	0.29	74	1.64	0.30	0.32	0.57
High risk	1	1	24	1.65	0.07	27	1.59	0.07		-
FEP	2	2	59	2.10	0.43	37	2.06	0.43	0.18	0.67
Chronic	2	2	46	1.48	0.57	10	1.40	0.58		-
Medicated	2	2	22	1.35	0.37				1.04	0.31
Medication-free	2	2	22	1.25	0.36					

Table 2 Multicentre data for PANSS and CPZ dose equivalents in schizophrenia patients (excluding high risk subjects).
Linear mixed-effects model analyses assessed the relation between glutamate measures and PANSS and CPZ dose equivalents. "NS" denotes no significant correlation between glutamatergic metabolites and PANSS positive, negative and general scores. "-" indicates values were only available for one study.

Metabolite	PANSS sub scales	# Studies	# Sites	PANSS	Sig	# Studies	# Sites	CPZ	Sig	
				n	*P*			*n*	F	*P*
Medial Frontal Cortex										
Glutamate	All PANSS sub-scales	3	2	64	NS	2	2	35	3.704	0.06
Glx		3	2	64	NS	1	1	14	-	-
Glutamine		3	1	13	NS	2	2	27	1.245	0.28
Thalamus										
Glutamate	All PANSS sub-scales	2	1	18	NS	2	2	30	0.576	0.45
Glx		2	1	21	NS	1	1	12	-	-
Striatum										
Glutamate	All PANSS sub-scales	2	1	42	NS	0	0	0	-	-
Glx/Cr		4	3	76	NS	1	1	15	-	-
Medial temporal Lobe										
Glx/Cr	PANSS Positive	3	2	93	0.09	2	2	43	0.664	0.42
	PANSS Negative	3	2	85	**0.003**					
	PANSS General	3	2	85	0.21					

2.3.2.1. Medial Frontal Cortex

Glutamate

In the medial frontal cortex, CSF-corrected glutamate measures were available from 5 studies (3 sites) of 206 cases (101 high risk, 48 FEP and 57 chronic schizophrenia patients) and 179 controls (Demjaha et al., 2014; Egerton et al., 2014; Natsubori et al., 2014; Theberge et al., 2002; Théberge et al., 2003). Glutamate concentrations did not significantly differ between cases and controls (see Table 1). When clinical groups were analysed separately, the high risk group (Egerton et al., 2014; Natsubori et al., 2014) and the FEP patient group (Egerton et al., 2014; Natsubori et al., 2014; Theberge et al., 2002) did not significantly differ from controls, but significantly lower glutamate levels were found in chronic patients relative to controls ($F(112)=4.154$, $P=0.044$; Figure 10) (Demjaha et al., 2014; Natsubori et al., 2014; Théberge et al., 2003).

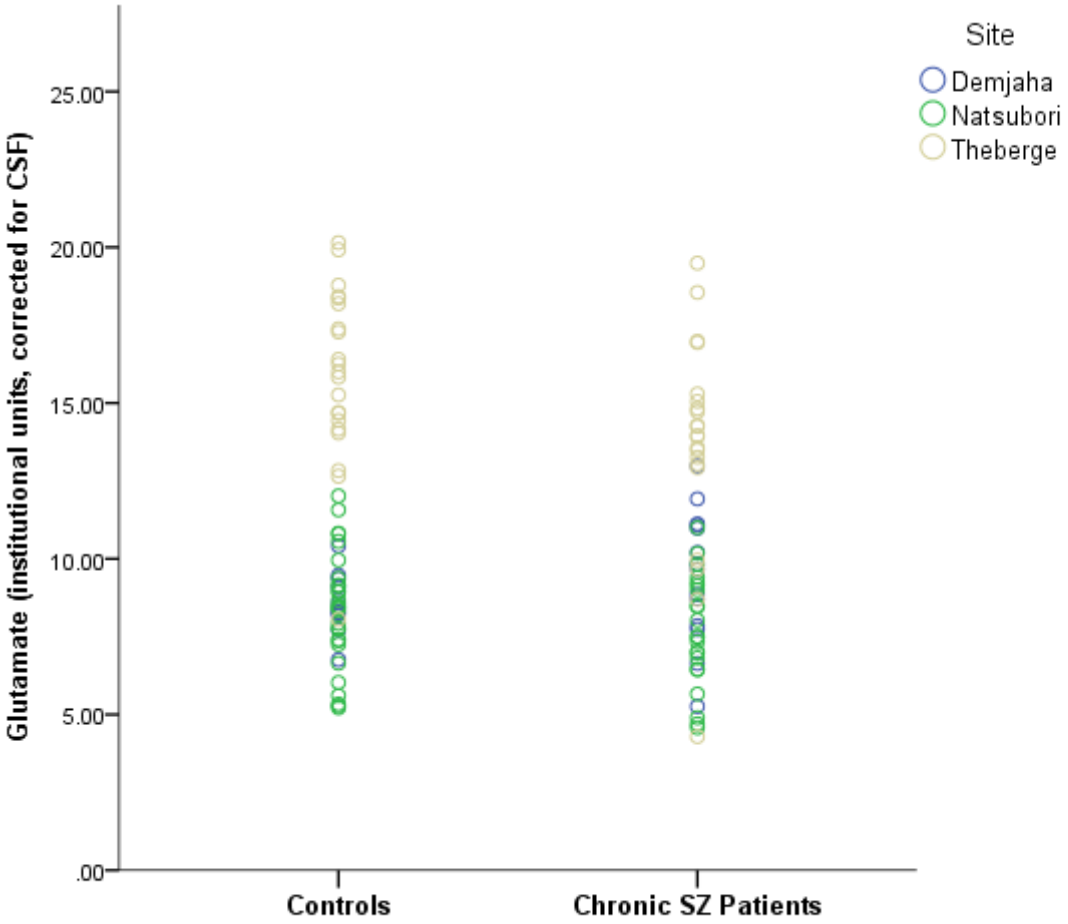

Figure 10 CSF-corrected glutamate (institutional units) in the medial frontal cortex in controls and chronic schizophrenia patient groups.

Glutamate levels were significantly lower in chronic schizophrenia patients than controls (P=0.044). Site is colour-coded. The index lists the authors of studies contributing data from each site.

When patients were categorised according to antipsychotic medication status, medication-free patients had significantly higher glutamate levels than medicated patients ($F(62)=8.012$, $P = 0.006$; Figure 11) (Demjaha et al., 2014; Egerton et al., 2014; Theberge et al., 2002; Théberge et al., 2003). (The Natsubori et al., 2014 study only examined medicated patients, and so was not included to avoid site bias). Furthermore, there was a trend for a negative relationship between glutamate levels and CPZ equivalent dose ($F(32)=3.704$, $P=0.063$; Figure 12) (Demjaha et al., 2014; Théberge et al., 2003). No significant association was found between CSF-corrected glutamate values and PANSS subscale scores (64 patients) (Demjaha et al., 2014; Egerton et al., 2014; Natsubori et al., 2014) (see Table 2).

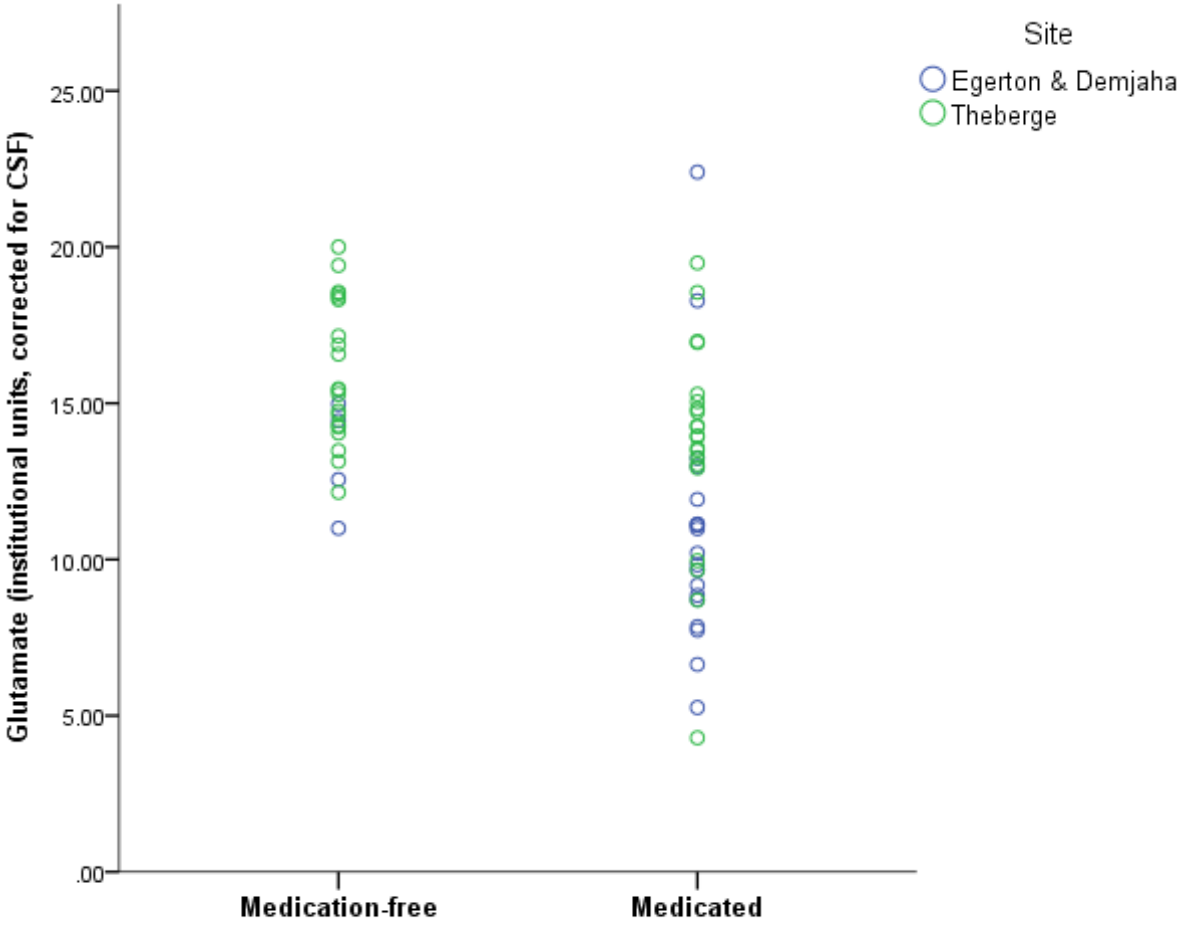

Figure 11 CSF-corrected glutamate (institutional units) in the medial frontal cortex in medication-free and medicated patient groups.

Glutamate levels were significantly higher in medication-free patients than controls (P=0.006). Site is colour-coded. The index lists the authors of studies contributing data from each site.

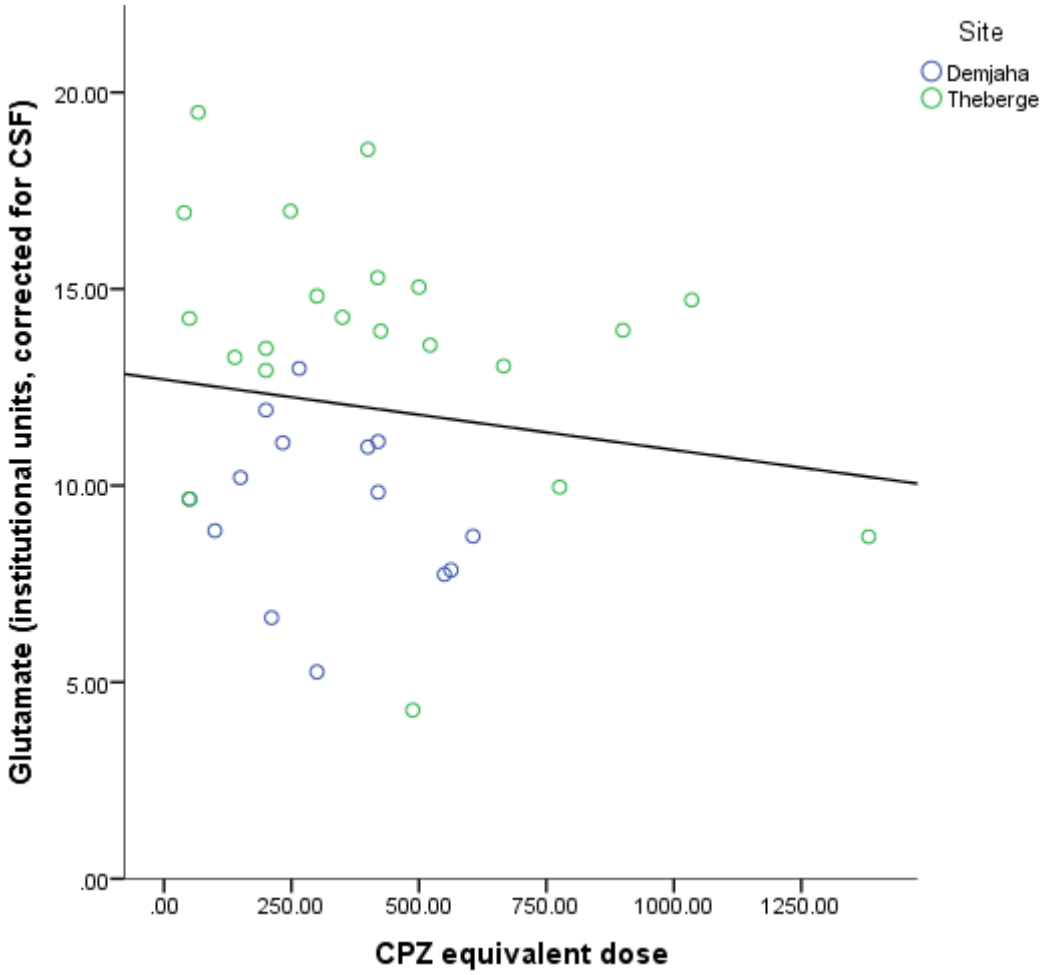

Figure 12 Trend for a negative correlation between CPZ equivalent dose and CSF-corrected glutamate (institutional units) in the medial frontal cortex of FEP and chronic schizophrenia subjects (P=0.063).

Site is colour-coded. The index lists the authors of studies contributing data from each site.

Glx

In the medial frontal cortex, CSF-corrected Glx measures were available from 3 studies (2 sites) of 170 cases (103 high risk, 28 FEP and 39 chronic schizophrenia patients) and 140 controls (Demjaha et al., 2014; Egerton et al., 2014; Natsubori et al., 2014). Glx concentrations did not significantly differ between cases and controls (see Table 1). When clinical groups were analysed separately, no significant difference relative to controls were found for high risk subjects (Egerton et al., 2014; Natsubori et al., 2014), FEP (Egerton et al., 2012; Natsubori et al., 2014) or chronic schizophrenia patients (Demjaha et al., 2014; Natsubori et al., 2014).

The effect of medication status and CPZ dose equivalents could not be assessed due to insufficient sample sizes. No significant association was found between CSF-corrected Glx values and PANSS positive, negative and general subscale scores (63 patients) (Demjaha et al., 2014; Egerton et al., 2012; Natsubori et al., 2014), see Table 2.

Glutamine

In the medial frontal cortex, CSF-corrected glutamine measures were available from 5 studies (2 sites) of 79 cases (25 high risk, 27 FEP and 27 chronic schizophrenia patients) and 63 controls (Demjaha et al., 2014; Egerton et al., 2014, 2012; Theberge et al., 2002; Théberge et al., 2003). Glutamine concentrations did not significantly differ between cases and controls (see Table 1). When clinical groups were analysed separately, a trend for higher glutamine levels were found in FEP patients than controls ($F(64)=3.323$, $P=0.073$; Figure 13) (Egerton et al., 2012; Theberge et al., 2002), whereas glutamine levels in chronic patients were lower than in controls ($F(48)=4.355$, $P=0.042$; Figure 14) (Demjaha et al., 2014; Théberge et al., 2003). Only one study in high risk subjects was available (Egerton et al., 2014).

When patients were categorised according to medication status, medication-free patients had significantly higher glutamine than medicated patients ($F(52)=4.724$, $P=0.034$; Figure 15) (Demjaha et al., 2014; Egerton et al., 2014, 2012; Theberge et al., 2002; Théberge et al., 2003). CPZ equivalent doses were not associated with glutamine (27 patients) (Demjaha et al., 2014; Théberge et al., 2003). No significant association was found between CSF-corrected glutamine values and PANSS positive, negative and general subscale scores (13 patients) (Demjaha et al., 2014; Egerton et al., 2014, 2012), see Table 2.

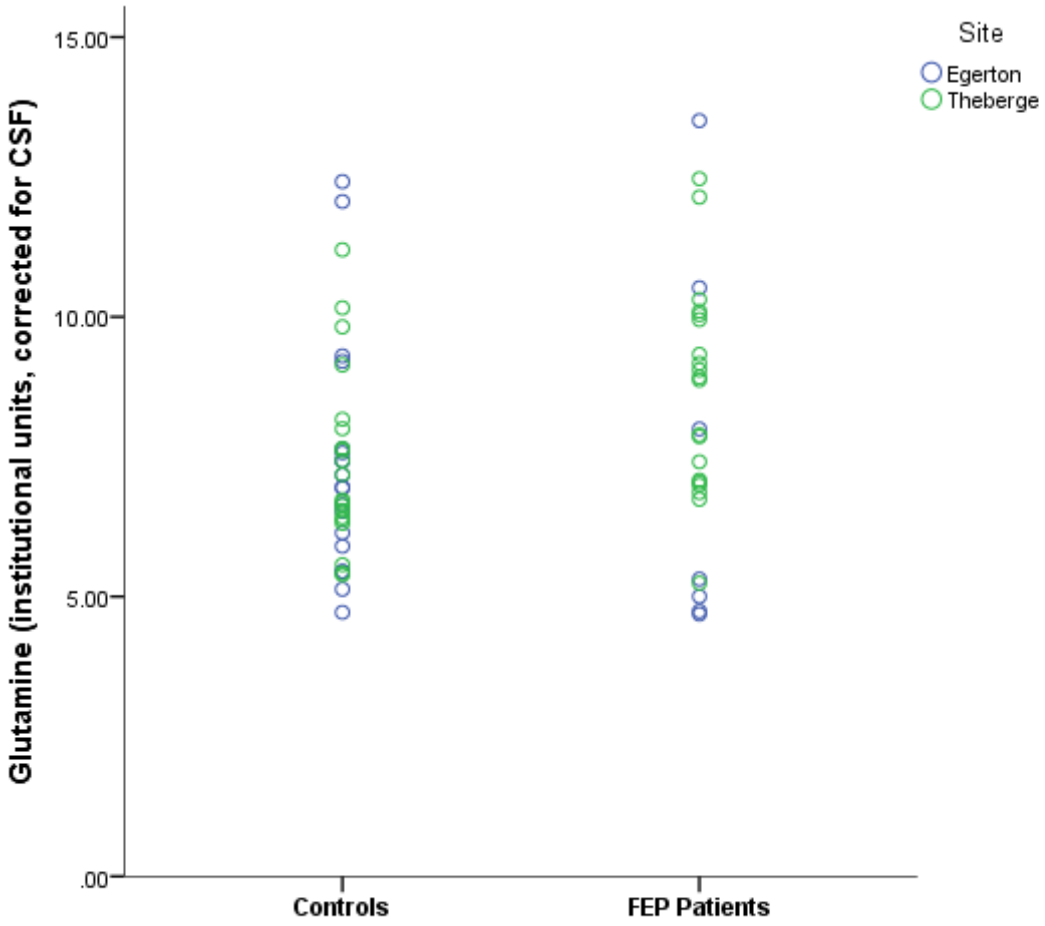

Figure 13 CSF-corrected glutamine (institutional units) in the medial frontal cortex in controls and first episode psychosis (FEP) patient groups.

Glutamine levels were significantly higher in FEP patients than controls (P=0.048). Site is colour-coded. The index lists the authors of studies contributing data from each site.

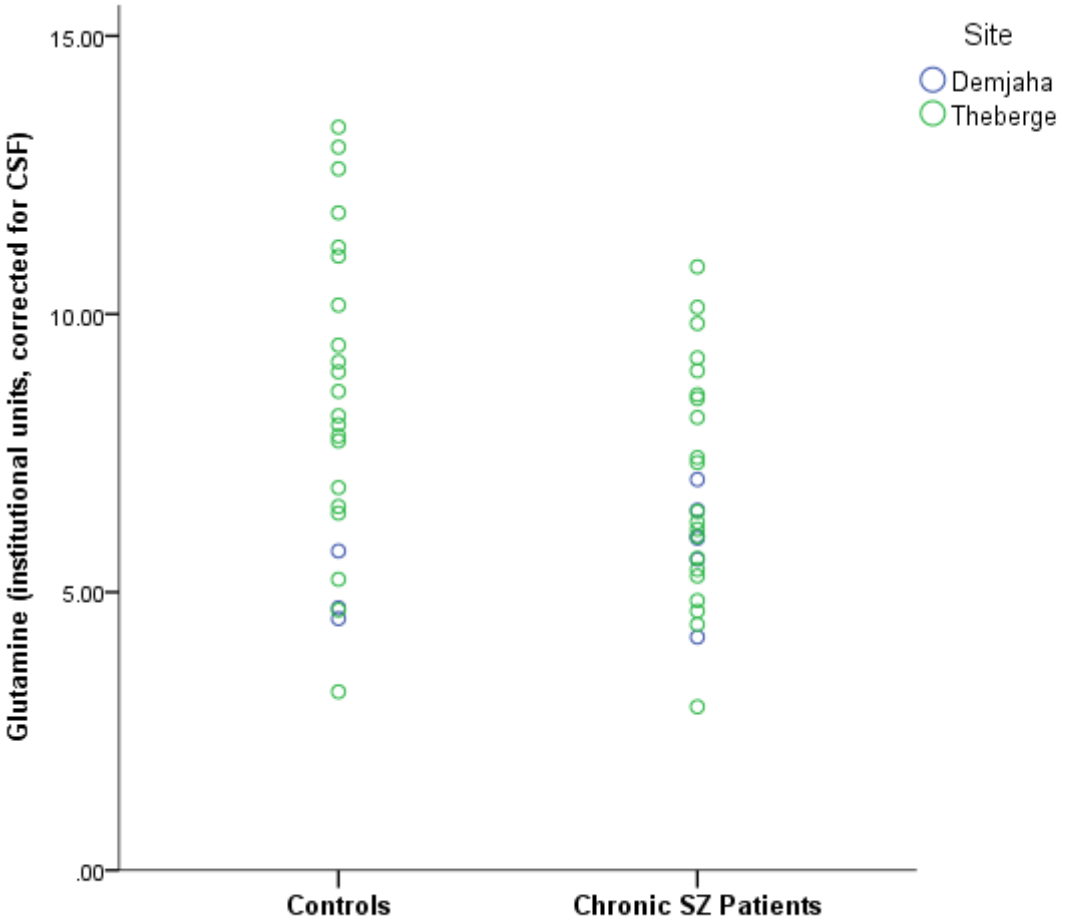

Figure 14 CSF-corrected glutamine (institutional units) in the medial frontal cortex in controls and chronic schizophrenia patient groups.

Glutamine levels were significantly lower in chronic schizophrenia patients than controls (P=0.042). Site is colour-coded. The index lists the authors of studies contributing data from each site.

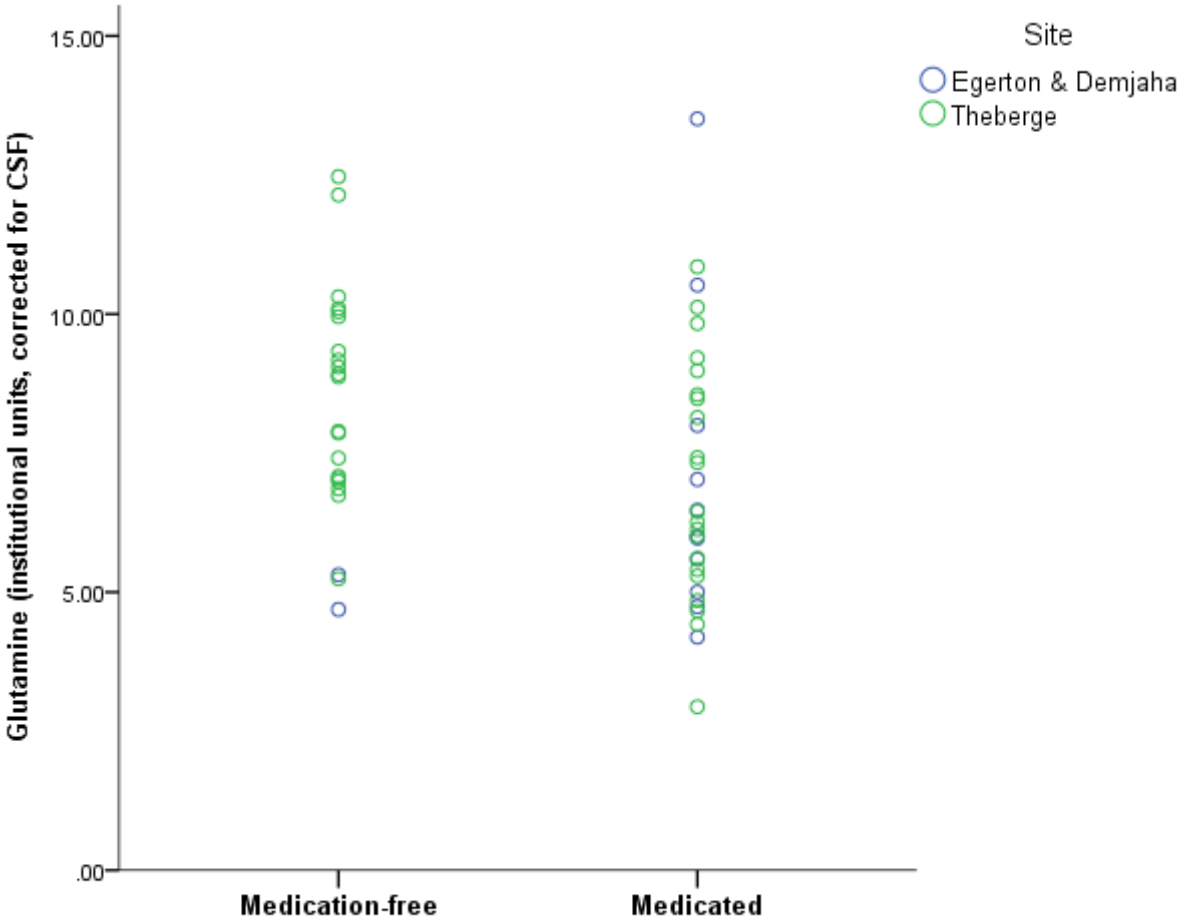

Figure 15 CSF-corrected glutamine (institutional units) in the medial frontal cortex in medication-free and medicated patient groups.

Glutamine levels were significantly higher in medication-free patients than controls (P=0.034). Site is colour-coded. The index lists the authors of studies contributing data from each site.

2.3.2.2. Thalamus

Glutamate

In the thalamus, CSF-corrected glutamate measures were available from 4 studies (2 sites) of 136 cases (80 high risk, 26 FEP and 30 chronic schizophrenia patients) and 105 controls (Demjaha et al., 2014; Egerton et al., 2014; Theberge et al., 2002; Théberge et al., 2003). Glutamate concentrations did not significantly differ between cases and controls (see Table 1). When clinical groups were analysed separately, no significant difference relative to controls were found for FEP (Egerton et al., 2014; Theberge et al., 2002) and chronic schizophrenia patients (Demjaha et al., 2014; Théberge et al., 2003). Only one study reported thalamic glutamate values in high risk subjects (Egerton et al., 2014).

Medication-free patients did not significantly differ from medicated patients (Demjaha et al., 2014; Egerton et al., 2014; Theberge et al., 2002; Théberge et al., 2003). No significant relationship was found between glutamate and PANSS positive, negative or general subscale scores (18 patients) and CPZ equivalent dose (30 patients) (Demjaha et al., 2014; Théberge et al., 2003), see Table 2.

Glx

CSF-corrected Glx measures were available from 2 studies (1 site) of 97 cases (76 high risk, 9 FEP and 12 chronic schizophrenia patients) and 65 controls (Demjaha et al., 2014; Egerton et al., 2014). Glx concentrations did not significantly differ between cases and controls (see Table 1). Clinical groups and medication status groups could not be analysed separately, as data was not available for more than one study.

No significant relationship was found between Glx and PANSS positive, negative or general subscale scores (21 cases) (Demjaha et al., 2014; Egerton et al., 2014). Only one study reported CPZ equivalent doses (Demjaha et al., 2014), see Table 2.

2.3.2.3. Striatum

Glutamate

In the striatum, CSF-corrected glutamate measures were available from 2 studies (1 site) of 60 cases (18 high risk, 42 FEP and 0 chronic schizophrenia patients) and 58 controls (de la Fuente-Sandoval et al., 2013, 2011). Glutamate concentrations were significantly higher in cases relative to controls ($F(116)=20.527$, $P=<0.001$; Figure 16). When clinical groups were analysed separately, significantly higher glutamate levels were found in FEP patients

(F(98)= 24.620, *P*=<0.001; Figure 17) (de la Fuente-Sandoval et al., 2013, 2011). Only one study reported striatal glutamate values in high risk subjects (de la Fuente-Sandoval et al., 2011). Medication-status could not be investigated as all patients were medication-free. No significant relationship was found between glutamate and PANSS positive, negative or general subscale scores (42 cases) (de la Fuente-Sandoval et al., 2013, 2011), see Table 2.

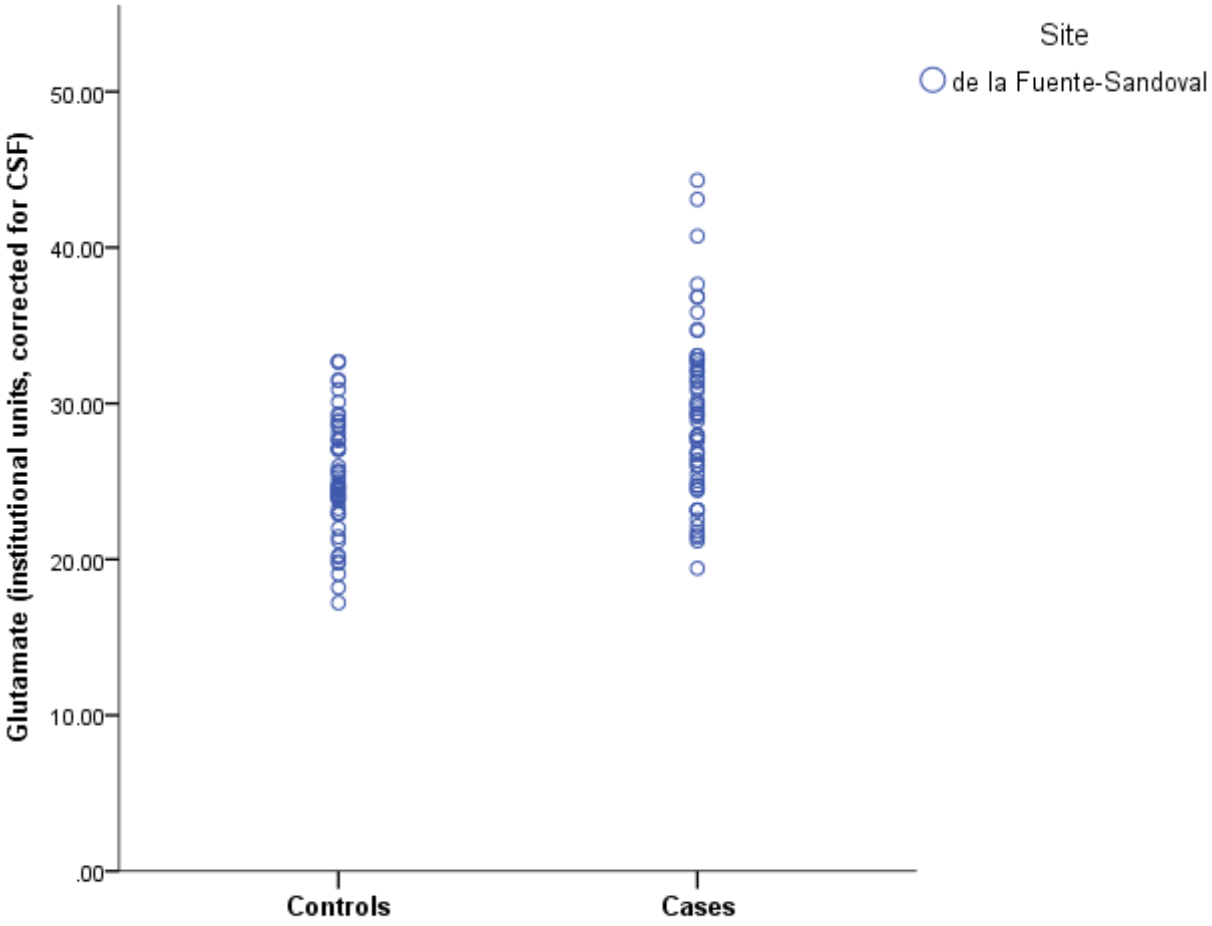

Figure 16 CSF-corrected glutamate (institutional units) in the striatum in controls and cases (high risk, FEP and chronic schizophrenia subjects).

Glutamate levels were significantly higher in cases than controls (*P*=<0.001). Site is colour-coded. The index lists the authors of studies contributing data from each site.

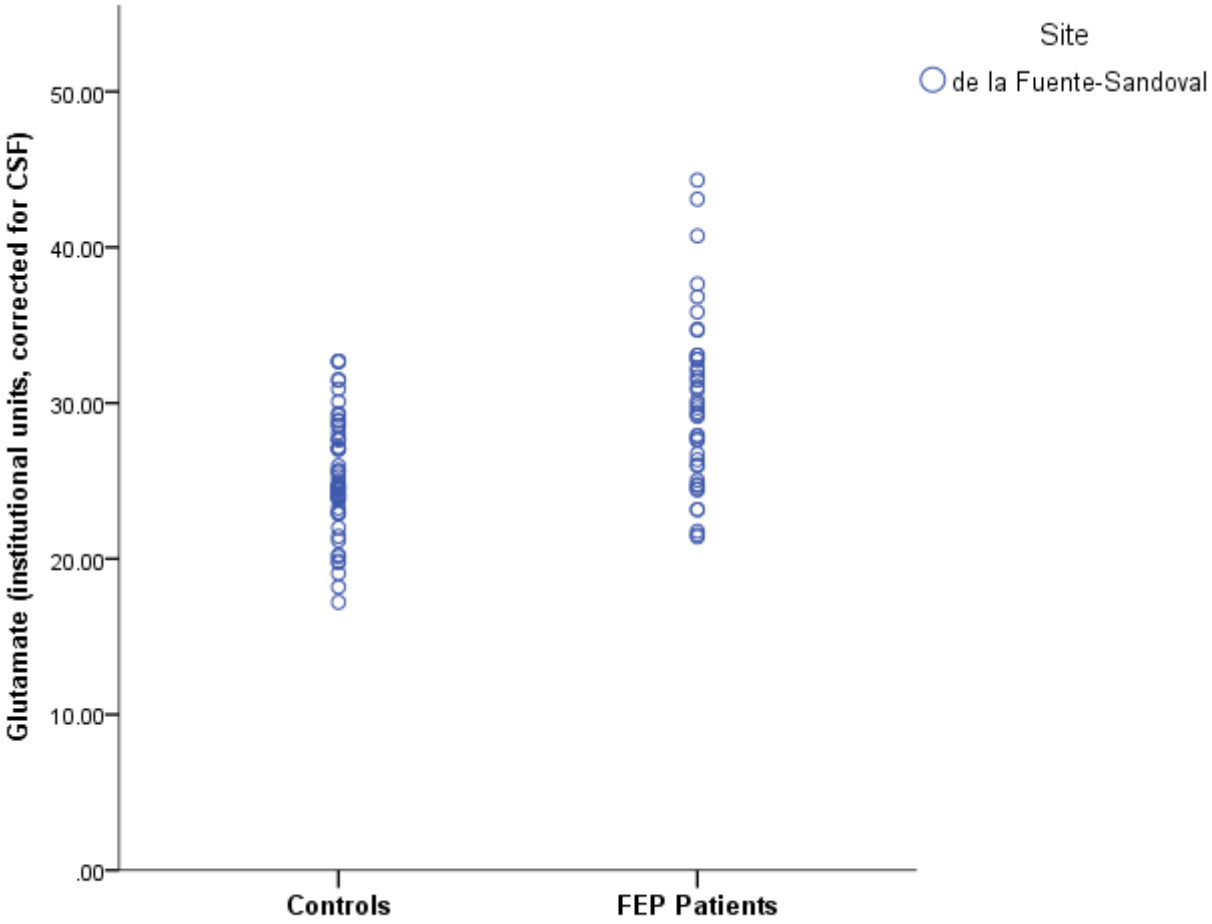

Figure 17 CSF-corrected glutamate (institutional units) in the striatum in controls and First Episode Psychosis patients.

Glutamate levels were significantly higher in FEP patients than controls (*P*=<0.001). Site is colour-coded. The index lists the authors of studies contributing data from each site.

Glx

Glx/Cr measures were available from 4 studies (3 sites) of 94 cases (18 high risk, 60 FEP and 16 chronic schizophrenia patients) and 91 controls (de la Fuente-Sandoval et al., 2013, 2011; Goto et al., 2012; Yamasue et al., 2003). Glx/Cr concentrations were significantly higher in cases relative to controls (F(181)=4.516, *P*=0.035; Figure 18). When clinical groups were analysed separately, no significant difference relative to controls were found for FEP patients (de la Fuente-Sandoval et al., 2013, 2011; Goto et al., 2012). Only one study reported striatal Glx/Cr values in high risk subjects (de la Fuente-Sandoval et al., 2011) and chronic schizophrenia patients (Yamasue et al., 2003). Medication-free patients did not

significantly differ from medicated patients, although it should be noted that medication status is not independent from site (de la Fuente-Sandoval et al., 2013, 2011; Goto et al., 2012; Yamasue et al., 2003). No significant relationship was found between glutamate and PANSS positive, negative or general subscale scores (76 cases) (de la Fuente-Sandoval et al., 2013, 2011; Goto et al., 2012; Yamasue et al., 2003), see Table 2.

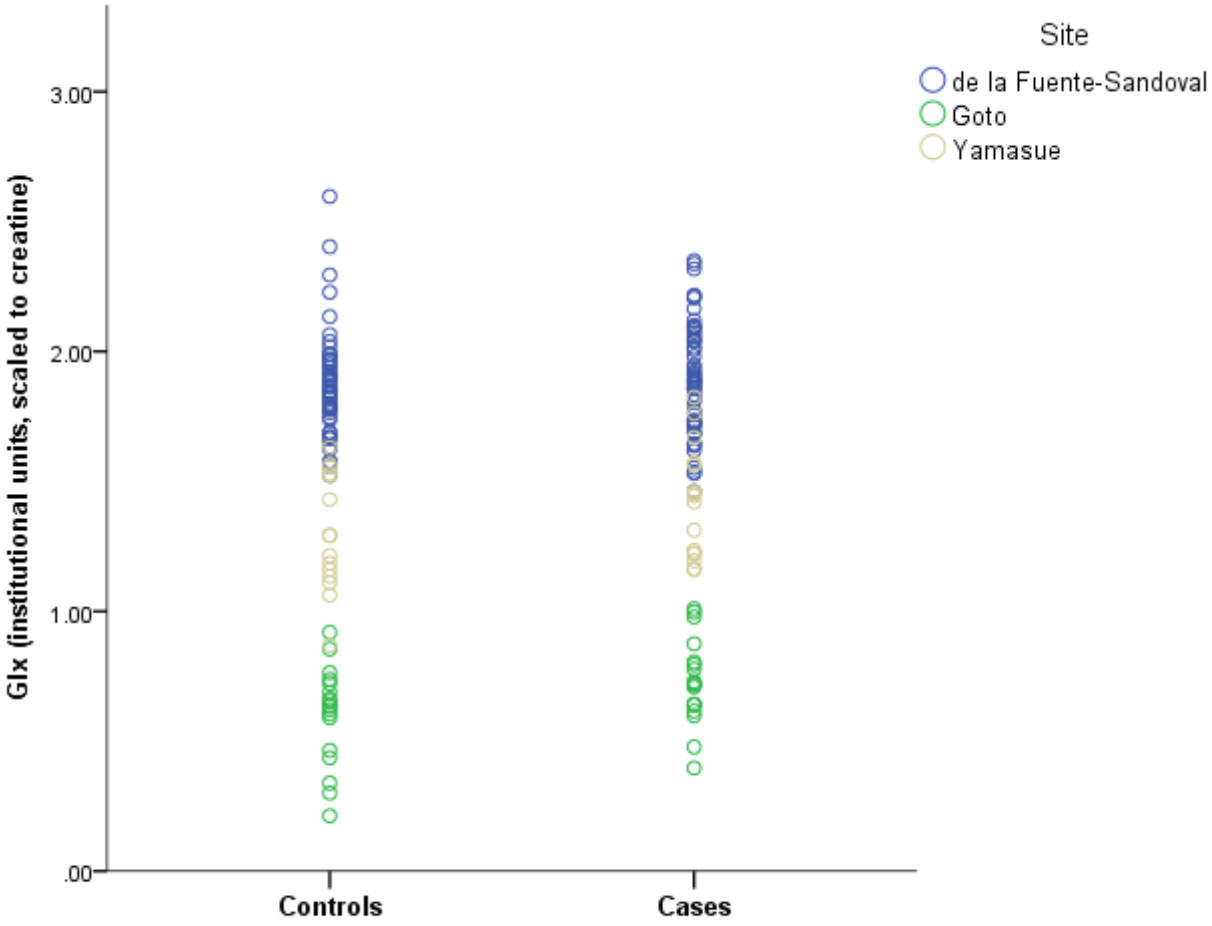

Figure 18 Glx/Cr (institutional units) in the striatum in controls and cases (high risk, FEP and chronic schizophrenia subjects).

Glx/Cr ratio was significantly higher in cases than controls (*P*=0.035). Site is colour-coded. The index lists the authors of studies contributing data from each site.

2.3.2.4. Medial temporal lobe

Glx

Glx/Cr measures were available from 5 studies (4 sites) of 129 cases (24 high risk, 59 FEP and 46 chronic schizophrenia patients) and 74 controls (Galińska et al., 2009; Lawrence S Kegeles et al., 2000; Stone et al., 2009; Szulc et al., 2011; Wood et al., 2008). Glx/Cr did not significantly differ between cases and controls (see Table 1). When clinical groups were analysed separately, no significant difference relative to controls were found for FEP patients (Galińska et al., 2009; Wood et al., 2008). Only one study reported Glx/Cr values in high risk subjects (Stone et al., 2009) and control subjects were not available at all sites for studies examining patients with chronic schizophrenia (Lawrence S Kegeles et al., 2000; Szulc et al., 2011). Medication-free patients did not significantly differ from medicated patients (Lawrence S Kegeles et al., 2000; Wood et al., 2008). Glx/Cr and PANSS negative scores were positively correlated, and survived correction for multiple comparisons ($F(82)=9.292$, $P=0.003$, 85 cases; Figure 19) (Galińska et al., 2009; Szulc et al., 2011; Wood et al., 2008). No significant relationship was found for PANSS positive (93 cases) or general (85 cases) subscale scores, or CPZ equivalent dose (59 cases) (Galińska et al., 2009; Wood et al., 2008), see Table 2.

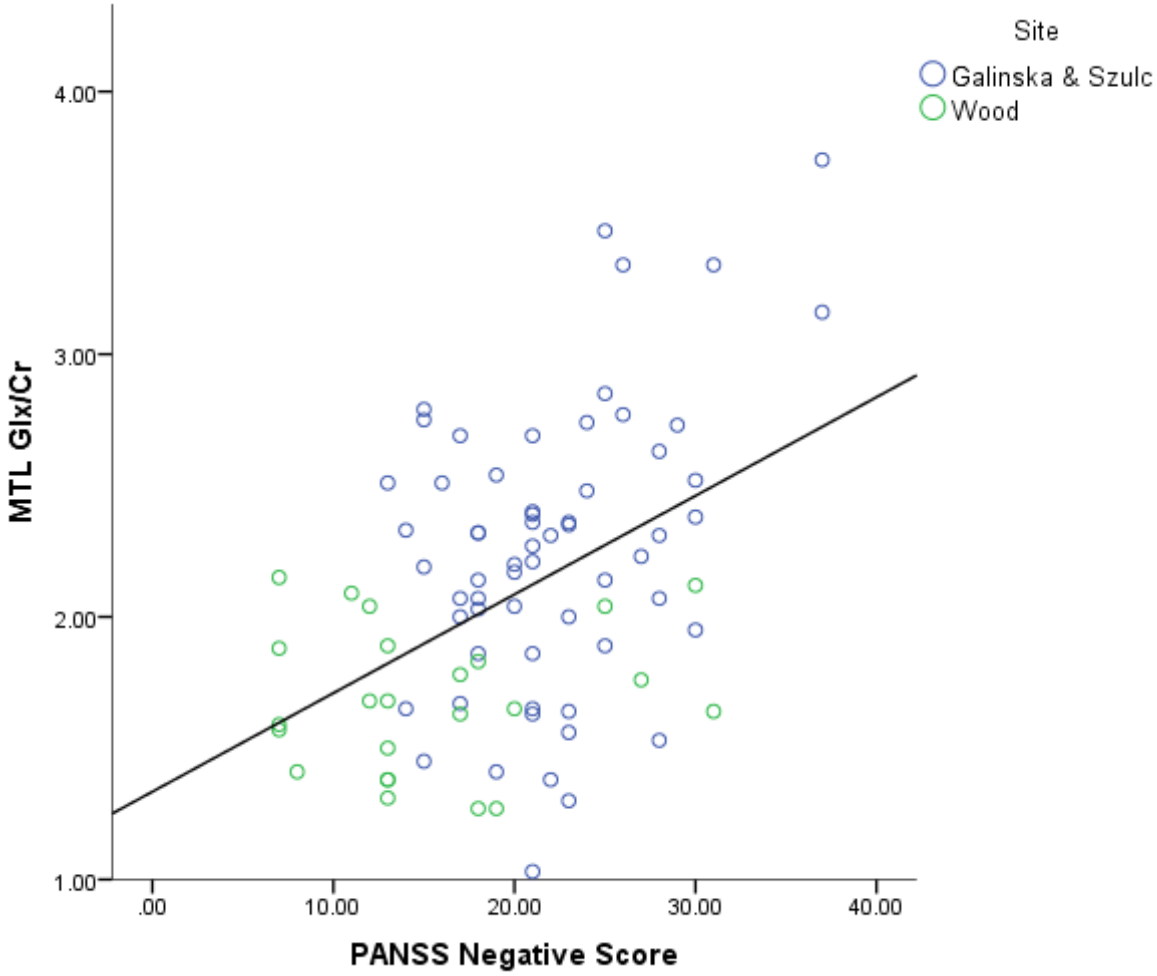

Figure 19 Positive correlation between PANSS negative score and Glx/Cr (institutional units) in the medial temporal lobe of FEP and chronic schizophrenia subjects (P=0.003).

Increasing PANSS score denotes more severe symptoms. Site is colour-coded. The index lists the authors of studies contributing data from each site.

2.3.3. Discussion

In the medial frontal cortex, glutamate and glutamine levels were lower in chronic patients than controls, and were lower in medicated patients than in patients who were medication-free. In addition there was a trend for a negative correlation between CPZ equivalent dose and glutamate, but not glutamine levels. However, all chronic patients were medicated and so these analyses cannot determine whether clinical stage or medication status play a role in lowered glutamate function.

For glutamine in the medial frontal cortex, a trend for elevated levels in FEP patients and a significant reduction in glutamine in chronic patients were strongly influenced by a single study which contributed the majority of data (Theberge et al., 2002; Théberge et al., 2003 respectively). Thus additional data is required for a true multicentre comparison. The finding of elevated glutamine in medication-free patients largely reflected the comparison of unmedicated FEP patients from Theberge et al., 2002 with medicated chronic patients from Theberge et al., 2003, and so additional data in medicated FEP/unmedicated chronic patients is needed to clarify the role of medication.

The trend for higher medial frontal glutamine in FEP patients than controls is consistent with the findings from the main meta-analysis (Chapter 2.2), although lower glutamine and glutamate were not detected in chronic patients. For Glx, no differences in the medial frontal cortex were found in FEP or chronic patient groups in both the present multicentre analysis and the main meta-analysis. However the meta-analysis found higher Glx levels in high risk subjects relative to controls. In the thalamus, the multicentre analysis did not detect any group differences for glutamate or Glx, however sufficient data were not available to examine glutamine, which was elevated in cases in the meta-analysis. It is of interest that Glx/Cr levels did not differ in the medial temporal lobe, as CSF-corrected Glx was elevated in both cases and chronic patients relative to controls in the meta-analysis. However, the multicentre analysis could not assess the chronic patient group separately due to an insufficient number of studies, and if effect-sizes were relatively large in this group, this could explain why significant differences were not detected in the cases overall.

In the striatum, both the multicentre analysis and the meta-analysis found elevated glutamate and Glx levels in cases. The striatum is a region that has only been recently studied using 1H-MRS, so separate analyses in clinical groups were not feasible for all glutamatergic metabolites. Higher glutamate in the multicentre analysis and higher Glx in the meta-analysis were detected in the striatum of FEP patients. Chronic patients could not

be examined separately in either the multicentre or meta-analysis, and future studies in this patient group will confirm whether striatal glutamate changes are specific to the initial stages of disease or are stable over time.

In summary, the multicentre analysis found altered glutamate metabolite levels in the medial frontal cortex and striatum of schizophrenia patients relative to controls. These findings are largely consistent with those of the meta-analysis, except that the multicentre analysis found lower medial frontal glutamate and glutamine in chronic patients. It is unclear whether the latter reflects an effect of illness stage or medication. For group comparisons, the meta-analysis had more statistical power than the multicentre analysis, as there were only a limited number of studies per region available for the latter. However, a multicentre analysis is better placed to assess the correlation between glutamate measures and symptom severity or CPZ dose equivalents. Overall, there was little evidence that PANSS symptom scores were related to glutamate metabolite levels, although increased Glx levels in the medial temporal lobe were correlated with the severity of negative symptoms. This finding in the MTL is in agreement with a SPET study which reported that reduced NMDAR binding in the hippocampus was associated with worsening negative symptoms (Pilowsky et al., 2006). The multicentre analysis was unable to examine how longitudinal changes in symptoms relate to changes in glutamate measures over time, which has been reported in a recent study (de la Fuente-Sandoval et al., 2013). As more longitudinal studies are conducted, sufficient data will become available for such analyses.

The use of CPZ equivalent doses to explore medication effects is of limited utility, as patient reports of medication history may be inaccurate, medication effects on glutamate may not be dose-dependent, and may vary with the type of antipsychotic used. A further caveat is that they do not account for variations in medication adherence. Scanning antipsychotic-naive first episode patients before and after the introduction of antipsychotic treatment would provide an ideal means of assessing 1) the effect of medication on glutamate metabolite levels, 2) the relationship between changes in symptom severity and longitudinal changes in glutamate measures and 3) the ability of glutamatergic measures to predict treatment response. I therefore adopted this experimental approach in the studies described in Chapter 4 and 5.

3. CHAPTER 3 – Reliability of longitudinal 1H-MRS measures of glutamate in healthy volunteers

3.1. Introduction

Longitudinal studies using serial 1H-MRS glutamate measurements must first ensure that these are reproducible and reliable. Reproducibility informs us of the precision of the mean within subject or between group measure, whereas reliability takes into account both within and between subject variation across the two timepoints (Bartlett and Frost, 2008). The test-retest reproducibility of 1H-MRS measures of glutamate concentrations and their reliability over short time periods have been assessed (see below), however the reliability of 1H-MRS glutamate measures over time periods exceeding two months, as would be generally required in a trial of antipsychotic treatment, remains to be reported.

Reproducibility and reliability are calculated by dividing the difference in a measure over two timepoints by the mean value across timepoints. Reproducibility applies the calculation to the whole sample, and can be reported as a measure of variance, or as the *mean* coefficient of variation (CV%). Reliability on the other hand, applies the calculation to each individual and the mean is taken; this is known as the *within-subjects* CV%.

Previous test-retest studies of 1H-MRS glutamate measurement error have reported reproducibility rather than reliability measures. The mean coefficient of variation (CV%) for glutamate and Glx is ~12%, measured in a number of brain regions, using different acquisition sequences, field strengths and test-retest time periods, see Table 3 (Bednařík et al., 2015; de la Fuente-Sandoval et al., 2013; Geurts et al., 2004; Hammen et al., 2005; Jang et al., 2005; Kaiser et al., 2005; Mullins et al., 2003; O'Gorman et al., 2011).

Few studies have examined the test-retest *reliability* of glutamate measures over time using single voxel 1H-MRS, see Table 4 (Bartha et al., 2000; Srinivasan, 2005; Taylor et al., 2010; Woermann et al., 1999). One study found a within-subjects CV of 8.9% for glutamate in the thalamus (Bartha et al., 2000) and another reported a within-subjects CV of 10.3% for glutamate in the parietal white matter (Srinivasan, 2005). However the test-retest scans in these studies were conducted within a few days of each other. Glutamate and Glx in the medial prefrontal cortex were found to be reliable over 7 days (within-subjects CV% < 7.4) (Taylor et al., 2010). One study investigated a longer time-frame of 1 day to 2 months, and reported that Glx measures were less reliable, with a within-subjects CV% of 35%

(Woermann et al., 1999). However this study examined the hippocampus, where it is difficult to acquire good quality spectra, and used a low field strength (1.5T) scanner.

A number of analyses can assess test–retest reliability. Pearson's product-moment correlation coefficient and intraclass correlation coefficient (ICC) are relative reliability measures which examine the consistency of an individual's position in a group. Pearson's *r* takes into account the linear relation of values regardless of the slope, and so does not provide information on the agreement of measures (Vaz et al., 2013). The intraclass correlation coefficient (ICC) measures the proportion of total variance that is due to differences between subjects and therefore its size depends on the variability in the sample. The within-subjects coefficient of variation is an absolute measure of reliability, which focuses on the degree to which individual measurements vary. The CV is most commonly reported in reliability studies of 1H-MRS measures and so will be reported here.

In order to inform the study design of a longitudinal 1H-MRS investigation of glutamate (Chapter 4), test-retest investigations using long-term inter-scan durations are needed. Schizophrenia is a relapsing-remitting disorder, and glutamate dysfunction may be specific to the stage of the disorder or the medication status of the patient. To investigate these effects, the 1H-MRS study (Chapter 4) proposes to scan patients at a long-term timepoint. In summary, this chapter will examine the feasibility of measuring 1H-MRS glutamate in healthy controls with an inter-scan duration of several months, in order to inform the longitudinal (10 month) 1H-MRS study in FEP patients.

The reliability estimates of glutamate, glutamine and Glx concentrations will inform which primary output measure will be used for the longitudinal (10 month) 1H-MRS study in FEP patients. This will take into account whether the correction method, commonly Cr-scaling or CSF-correction, affects the reliability of glutamate metabolite measures. In addition the reliability of glutamate measures using different versions of spectra quantification software will be assessed, as the most recent version of LCModel claims to better estimate metabolites with weaker signals, in particular glutamine.

Reproducibility								Mean CV%		
Study	Participants	Field	Sample *n* x *r*	Time between scans	Brain regions	Acquisition	Analysis method	Glu	Glx	Gln
Bednařík 2015	Healthy Volunteers	3	10 x 2	Same day	Hippocampus	semi-LASER	LCModel	5.9		23.5
de la Fuente-Sandoval 2013	Healthy Volunteers	3	18 x 2	4 weeks	Dorsal Caudate	PRESS	LCModel	3.8		
					Cerebellum			9.7		
Geurts 2004	Healthy Volunteers	1.5	10 x 2	1 day to 2 months	Parietal grey matter		LCModel	8.6		22
			10 x 2		Hippocampus			37		54
			10 x 2		Thalamus			21		71
Hammen 2005	Healthy Volunteers	1.5	15 x 2	4–6 weeks	Hippocampus	PRESS	LCModel		19.4	
Jang 2005	Healthy Volunteers	1.5	21 x 2	Within-session	ACC	PRESS	LCModel	11	7.2	
Kaiser 2005	Healthy Volunteers	4	10 x 2	7 days	Corona radiata	STEAM	Other	13.4		44.5
					Mesial motor cortex			11.6		20.5
Mullins 2003	Schizophrenia	1.5	12 x 2	7 days	Frontal white matter		LCModel		15	
					Caudate nucleus				24	
O'Gorman 2011	Healthy Volunteers	3	14 x 4	Within-session	DLPFC	MEGA-PRESS	LCModel	8	6	

Table 3 Previous reproducibility studies of 1H-MRS glutamate (Glu), Glx and glutamine (Gln) measures, assessed by mean percentage coefficient of variation (CV%).
Sample: n=number of individuals scanned; r=number of replicate scans acquired per individual.

Reliability

Study	Participants	Field	Sample *n* x *r*	Time between scans	Brain regions	Acquisition	Analysis method	Within-subjects CV%		
								Glu	Glx	Gln
Bartha 2000	Healthy Volunteers	1.5	10 x 2	Same day	Thalamus	STEAM	Other	8.9		16.9
Srinivasan 2005	Healthy Volunteers	3	16 x 2	Days (not specified)	Parietal white matter	PRESS	LCModel	10.3		
Woermann 1999	Healthy Volunteers	1.5	7 x 2	1 day to 2 months	Hippocampus	PRESS	LCModel		35	
Taylor 2010	Healthy Volunteers	3	10 x 2	7 days	Medial prefrontal cortex	PRESS	LCModel	7.4	6.8	

Table 4 Previous reliability studies of 1H-MRS glutamate (Glu), Glx and glutamine (Gln) measures, assessed by mean percentage coefficient of variation (CV%).
Sample: n=number of individuals scanned; r=number of replicate scans acquired per individual.

3.2. Method

3.2.1. Study objectives

1. Assess the test-retest reproducibility and reliability of longitudinal 1H-MRS glutamate measures.
2. On the basis of the above, select the LCModel software version giving the highest reliability and reproducibility of these measures.
3. Investigate the effect of Cr-scaling and CSF-correction on glutamate reliability estimates.
4. Perform a power analysis for the longitudinal 1H-MRS study.

3.2.2. Ethical approval, data protection, informed consent and confidentiality

Healthy control data were used from an ongoing study by A Egerton and J Stone, as part of a larger study comparing healthy controls with at risk mental health subjects (Egerton et al., 2014; Stone et al., 2009). The study was granted ethical approval by the Institute of Psychiatry Ethics Committee/South London and Maudsley NHS Trust Ethics Committee. All data was stored according to the Data Protection Act 1998, under anonymous identification numbers in locked storage and password protected documents.

Healthy controls were obtained through email advertisements. Subjects were provided with written study information, and the study was explained in person, whereupon subjects were invited to give written consent. Subjects were aware that they could withdraw at any time, and that their details were confidential. Subjects agreed to their GP being contacted if medically significant results required further investigation.

3.2.3. Sample

Subjects were aged 18-40, right-handed and had no MRI contraindications and no previous diagnosis of a psychiatric disorder.

32 subjects (mean age 24.4 years, SD 4.3; 15 males, 17 females) were scanned twice, with a mean elapsed time of 17 months (SD 6.78). This inter-scan interval is similar to that which will be used in the longitudinal 1H-MRS study in FEP (see Chapter 4).

3.2.4. 1H-MRS protocol

All investigations were performed on a 3 Tesla General Electric (Milwaukee, Wisconsin) MRI scanner. The scan acquisition and analysis parameters match those used in the longitudinal 1H-MRS study in FEP (see Chapter 4).

An initial localiser scan was used to identify the anterior commissure-posterior commissure line (AC-PC) and interhemispheric angle. Structural images were acquired using an axial 2D T2-weighted Fast Spin Echo scan and an axial fast fluid-attenuated inversion recovery (FLAIR) scan. A whole brain 3D coronal IR-SPGR (inversion recovery prepared spoiled gradient echo) scan was obtained to localise 1H-MRS voxels, giving isotropic 1.1-mm voxels in a scan time of approximately 6 min (echo time (TE) = 2.82 msec; repetition time (TR) = 6.96 msec; inversion time = 450 msec; excitation flip angle = 20°).

1H-MRS spectra were acquired using a PRESS sequence (Point RESolved Spectroscopy), echo time TE=30msec; repetition time TR=3000msec, 96 averages. The standard GE PROton Brain Examination (PROBE) sequence was used to supress water signals via a standardised chemically selective suppression (CHESS) water suppression routine. Auto pre-scans were performed twice before each scan to optimise water suppression and shimming. Spectra were collected in left thalamus, where the ROI has a voxel resolution of 15 (right-left) x 20 (anterior-posterior) x 20mm (superior-inferior), localised from sagittal and coronal localisers where the thalamus is widest with the least CSF contamination, obtaining a minimum water linewidth (full-width half maximum) of 10Hz after shimming. Spectra were also obtained from the anterior cingulate, localised from the midline sagittal localizer, with the centre of the 20 x 20 x 20mm ROI being placed 13mm above the anterior portion of the genu of the corpus callosum, 90º to the anterior commissure-posterior commissure line (AC-PC), and making sure to avoid the corpus callosum. A minimum water linewidth (FWHM) below 7Hz was obtained after shimming. A signal-to-noise ratio (S/N) ≥8 and linewidth of <0.1 ppm were required for inclusion.

After subject scanning, PRESS spectra from a phantom containing standardised concentrations of brain metabolites (Provencher, 1993) were obtained to assess scanner drift or step-changes over the study.

3.2.5. 1H-MRS analysis

LCModel version 6.1-4F and version 6.3-0I (Provencher, 2015, 1993) were used to estimate the concentration of metabolites, by fitting the output to a basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, Gln, glutamate,

glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, and taurine), acquired with the same field strength (3 Tesla), localization sequence (PRESS), and echo time (30 msec) (Provencher, 2015). Poorly fitted metabolite peaks (Cramer–Rao minimum variance bounds (CRVB) >20% as reported by LCModel) were excluded from further analysis. LCM produces water-scaled values that are presented as 'institutional units'. Metabolites are also reported as a concentration ratio to creatine.

Water-scaled metabolite concentrations (institutional units) were CSF-corrected. Cerebrospinal fluid (CSF) contains lower metabolite concentrations and so large volumes of CSF in the ROI would underestimate the metabolite value in the specified brain region. CSF-correction was carried out by segmenting IR-SPGR 3D images into grey, white and CSF volumes using Statistical Parametric Mapping 5 (SPM5; Wellcome Department of Imaging Neurosciences, University College London, UK). ROI co-ordinates were obtained from spectra file headers using General Electric's spectroscopy processing tool Sage, to obtain brain tissue volumes from the same region in the IR-SPGR images. Grey matter and white matter volume in the ROI are added, and this value is used to correct metabolite values using the following calculation:

Uncorrected metabolite x [vol + 1.55 x (1 - vol)] / vol

Vol = grey matter + white matter

The inclusion of "1.55" in the above equation is to correct for the estimated water concentration of the voxel for partial volume CSF contamination, as the spectra are water-scaled (this assumes a CSF water concentration of 55,556 mol/m3 and the LCModel default brain water concentration of 35,880 mol/m3).

3.2.6. Statistical analysis of reproducibility and reliability

CSF-corrected and Cr-scaled concentrations of glutamate, glutamine, and Glx in the ACC and thalamus at both timepoints, were entered into Microsoft Excel spreadsheets to perform the following reproducibility and reliability calculations:

VAR% was used to test the reproducibility of metabolite measures between the baseline and follow-up scan with the following calculation:

(retest mean value – test mean value)/ 0.5 (test mean value + retest mean value) × 100

VAR% is not a measure of reliability as it does take not into account individual subject variance, unlike within-subject CV%.

The within-subject percentage coefficient of variation (CV%) was used to test the reliability of metabolite measures between the baseline and follow-up scan.

The within-subject CV% was calculated using the root mean square approach, as an alternative to the logarithmic method (http://www-users.york.ac.uk/~mb55/meas/cv.htm)

Within subject variance: $s = (test-retest)^2/2$

Subject mean: $m = (test+retest)/2$

CV squared: $s2m2 = s/m^2$

CV% = √means2m2 x 100

Power calculations were performed using 'PS' Software (Dupont and Plummer, 1990) at an alpha level of .05 and power of .80. An ability to detect a 15% change in the mean glutamate level in the anterior cingulate between patients and controls was chosen based on a previous 3T study, which reported a 16% change in Glu/Cr ratios in the anterior cingulate between symptomatic schizophrenia patients and those in remission (Egerton et al., 2012).

3.3. Results

Results are listed in Table 5. In the ACC, within-subjects CV% values were between 10% and 17% and variance (VAR%) was below 10% for glutamate, glutamine and Glx values. In the thalamus, within-subjects CV% values were between 16% and 23% and variance (VAR%) was below 20% for glutamate and Glx values. Glutamine could not be resolved in the thalamus at both timepoints in any subject. The reliability (within-subjects CV% of ~20%) and reproducibility (variance of <20%) was higher than that reported in previous studies that examined reliability over an inter-scan interval of days (Bartha et al., 2000; Srinivasan, 2005; Taylor et al., 2010), probably due to the increased test-retest time interval, but was lower than a study examining reliability over 1 day to 2 months (Woermann et al., 1999).

More reliable measures from the ACC in comparison to the thalamus were expected, as the structure of the thalamus is more homogeneous and thus is harder to acquire 1H-MRS data from. No significant differences between timepoints were found using paired t-tests, except for CSF-corrected Glx levels in the thalamus using LCModel version 6.3-0I which approached significance (P=0.052). However, the within-subjects CV% is a more suitable statistical assessment for determining the reliability of measures. Any variation between repeated measurements is unlikely to be due to variation in the manual placement of voxel regions of interest, as low within-subjects CV% were found for ACC white plus grey matter (7%), ACC CSF (17%) and thalamus white plus grey matter (5%). As the amount of CSF is negligible in the thalamic voxel, it is not appropriate to conduct within-subjects CV% calculations, as structural imaging software cannot accurately measure the degree of CSF at such low concentrations. In addition no significant differences in the volume of CSF or white matter plus grey matter were found using paired t-tests.

When comparing metabolite correction methods, VAR% and CV% were comparable but somewhat lower for creatine (Cr)-scaled values than CSF-corrected values. Despite being reliable, Cr-scaled values may not be a suitable output measure for the longitudinal 1H-MRS study in FEP, as creatine concentrations may change with antipsychotic treatment. Therefore, CSF-corrected values will be used as the primary output measure, as this correction method still shows good reliability and reproducibility.

For the analysis software, LCModel 6.3-0I returned generally lower SD and CV%, but higher VAR% values than LCModel 6.1-4F. Version 6.3-0I also returned acceptable fits for glutamine in a higher number of subjects than version 6.1-4F (3 subjects using 6.1-4F in comparison to 13 subjects using 6.3-0I in the ACC). The low CV% values achieved with

LCModel 6.3-0I indicates good within subject reliability, and so will be suitable for the longitudinal study in FEP examining glutamate metabolite changes with antipsychotic treatment. Low VAR% values are less important as they indicate the precision of the mean between group measures but not within-subject reliability.

For CSF-corrected values obtained using LCModel 6.3-0I, both glutamate and Glx show good reliability and reproducibility, with Glx showing slightly superior CV% and glutamate showing slightly superior VAR%. As the longitudinal study will be conducted at 3T, Glx is the most suitable primary output measure, as glutamate and glutamine peaks overlap by <30% in the 2.25–2.55 ppm range (Snyder and Wilman, 2010a).

A power analysis at an alpha level of .05 and power of .80, indicated that sample sizes of 23 patients and 23 controls are required to detect a 15% difference in CSF-corrected Glx levels in the ACC, and 32 patients and 30 controls to detect a 15% difference in Glx levels in the thalamus.

For longitudinal analyses, the power analysis at an alpha level of .05 and power of .80, indicated that 13 patients are required to detect a 15% within-subjects difference in Glx levels in the ACC, and 17 patients to detect a 15% within-subjects difference in Glx levels in the thalamus.

Table 5 (overleaf) Reproducibility (VAR%) and reliability (CV%) of 1H-MRS measures of glutamate (glu), Glx and glutamine (Gln) in healthy controls.
See next page. Both Creatine-scaled (Cr) and CSF-corrected measures are reported. SD = standard deviation, CV = % Coefficient of Variation, VAR% = Variance. Sample size indicates power analysis calculations for between-subjects and within-subjects design to detect a 15% difference.

LCModel 6.1-4F

Brain region	Metabolite	Corr	*n*	Baseline Mean	Baseline SD	Follow-up Mean	Follow-up SD	CV %	VAR %	Sample size Between	Sample size Within
Anterior cingulate	Glu	CSF	31	13.3	2.6	13.2	2.4	15.8	0.8	26	14
	Glx	CSF	31	18.2	4	17.9	3.8	16.6	1.7	34	18
	Gln	CSF	3	8.4	3.6	9.3	4.1	9.8	10.2	131	67
	Glu	Cr	31	1.35	0.2	1.37	0.21	13.7	1.5	18	10
	Glx	Cr	31	1.85	0.38	1.85	0.31	16.4	0.0	26	14
	Gln	Cr	3	0.82	0.35	0.75	0.28	13.4	8.9	113	58
Thalamus	Glu	CSF	30	7.4	1.5	7.0	1.6	21.5	7.6	33	18
	Glx	CSF	30	9.2	2.6	8.4	1.8	20.3	9.2	45	24
	Glu	Cr	30	1.03	0.16	1	0.23	23.0	18.2	29	16
	Glx	Cr	30	1.27	0.3	1.23	0.28	22.8	18.3	38	20

LCModel 6.3-0I

Brain region	Metabolite	Corr	*n*	Baseline Mean	Baseline SD	Follow-up Mean	Follow-up SD	CV %	VAR %	Sample size Between	Sample size Within
Anterior cingulate	Glu	CSF	32	13.9	2.4	13.5	2.2	14.7	2.9	20	12
	Glx	CSF	32	19.7	3.4	19.1	3.5	14.3	3.1	23	13
	Gln	CSF	13	7.6	2.3	7.3	2.3	14.6	4.0	65	34
	Glu	Cr	32	1.34	0.17	1.34	0.19	12.3	0.0	14	8
	Glx	Cr	32	1.91	0.28	1.88	0.28	14.3	1.6	16	9
	Gln	Cr	13	0.73	0.19	0.67	0.18	15.8	8.6	48	25
Thalamus	Glu	CSF	31	7.8	1.6	7.3	1.5	19.0	6.6	30	16
	Glx	CSF	31	9.9	2.4	9	1.6	18.1	9.5	32	17
	Glu	Cr	31	1.05	0.16	1.03	0.21	16.0	1.9	23	13
	Glx	Cr	31	1.32	0.27	1.27	0.25	16.6	3.9	28	15

3.4. Discussion

In this study the test-retest reproducibility (VAR%) and reliability (within-subjects CV%) of glutamate measurements over a period of several months was estimated, comparing two correction methods, and two versions of the LCM analysis software. The results indicate that glutamate measures have a good reproducibility and reliability over long time periods.

This study was conducted to inform the primary outcome measure and sample size needed for a longitudinal investigation of 1H-MRS glutamate measures and the acute and medium-term response to antipsychotic medication (Chapter 4). The reliability of glutamate measures were examined in the same brain regions over a similar follow-up timescale to the proposed study.

Based on the present findings, CSF-corrected Glx has been selected as the primary outcome measure for the longitudinal study. Glx was selected, as although the reliability of glutamate and Glx was comparable, Glx is the more accurate signal, as 10% of the glutamate signal is contaminated by glutamine at 3T (Snyder and Wilman, 2010b). Glutamine is not an appropriate outcome measure as it was not resolved in the thalamus, and was only acquired in half of the sample for the ACC. The latest version of LCModel (6.3-0I) has been selected for the analysis of this data, as this was found to be more reliable than the previous version, likely due to improved fitting of spectra to the basis set.

In earlier 1H-MRS studies, metabolites were typically scaled to creatine as it was thought that creatine levels are relatively stable in the brain. However, creatine differences between patients with schizophrenia and healthy controls have been reported, as well as changes with age (Tibbo et al., 2013). Thus there has been a shift in reporting practices as CSF-corrected metabolite values are thought to be more reliable than Cr-scaled values. Therefore it is of interest that Cr-scaled values were found to be slightly more reliable than CSF-corrected values in this analysis. However CSF-correction rather than Cr-scaling will be used in the longitudinal study, as medication may affect creatine levels.

At least 17 patients will be required in the proposed longitudinal study (Chapter 4) in order to detect a 16% difference in Glx levels over time, as indicated by a power analysis of the selected outcome measure (CSF-corrected Glx, LCModel 6.3-0I).

CHAPTER 4 – Longitudinal investigation of 1H-MRS glutamate measures and the acute and medium-term response to antipsychotic medication.

4.1. Introduction and Study Objectives

This chapter outlines the study design and findings of a longitudinal 1H-MRS study that aims to determine the relationship between glutamate measures and the acute and medium-term response to antipsychotic medication.

The meta-analysis in Chapter 2 found that schizophrenia is associated with elevations in glutamatergic metabolites. Glutamate levels have also been found to differentiate patients based on their clinical response, as evidenced by three previous studies comparing responders and non-responders to treatment (Demjaha et al., 2014; Egerton et al., 2012; Mouchlianitis et al., 2015). However, as these studies are cross sectional, it is not known whether between-group differences are present at the first onset of psychosis and therefore predict treatment response, or whether effective treatment is associated with a reduction in glutamate.

To determine this, the present study examined glutamate measures in antipsychotic naive patients experiencing their first episode of psychosis and followed-up patients at a short (5 weeks) and medium-term (10 months) timepoint following treatment with antipsychotic medication. A medium-term timepoint was added as response to medication and medication-effects on glutamatergic metabolites may take longer than 5 weeks to occur. The main output measure was CSF-corrected Glx levels, as informed by the reliability and reproducibility analysis of glutamate measures outlined in Chapter 3. Glutamate measures were obtained in the ACC and thalamus, consistent with previous studies comparing responder and non-responder groups (Demjaha et al., 2014; Egerton et al., 2012; Mouchlianitis et al., 2015), as NMDAR antagonist administration to healthy volunteers increases metabolism in these regions (Deakin et al., 2008; De Simoni et al., 2013; Holcomb et al., 2005, 2001) and increases glutamate and glutamine concentrations in the ACC (Rowland et al., 2005; Stone et al., 2012). The overall objectives of the study are as follows:

Patients vs controls:

- To identify differences in Glx between patients and controls.

Responders versus non-responders to antipsychotic treatment:

- To investigate whether Glx levels at baseline predict response to 5 weeks of antipsychotic treatment.
- To investigate whether Glx declines with 5 weeks of treatment in responders but not in non-responders.
- To investigate the effect of medication on group differences in Glx by controlling for medication adherence.

Remission vs non-remission:

- To investigate whether Glx levels at baseline predict remission at a 10 month follow up.
- To investigate whether Glx declines over time in remission but not in non-remission patients.
- To investigate the effect of medication on group differences in Glx by controlling for medication adherence.
- To investigate the relationship between longitudinal change in Glx and the change in symptom severity over time.

4.2. Study design

Subjects were recruited from two studies: 15 patients were recruited from the OPTiMiSE clinical trial that involved a standardised treatment (amisulpride) and regular research assessments, and 6 patients were recruited from a study following a naturalistic treatment by clinicians with assessments limited to when subjects were scanned (TreatFEP study).

Ethical approvals

Both studies were granted ethical approval by the Institute of Psychiatry Ethics Committee/South London and Maudsley NHS Trust Ethics Committee. All data was stored according to the Data Protection Act 1998, under anonymous identification numbers in locked storage and password protected documents.

4.3. Participants

4.3.1. Patient Recruitment

Based on the reliability analysis from Chapter 3, 17 patients would provide adequate power to detect a 15% within-subjects difference in Glx levels in the thalamus, and 13 patients were required to detect a 15% within-subjects difference in Glx levels in the ACC between responders and non-responders.

Patients were recruited from Early Intervention teams from the South London and Maudsley NHS Trust.

Inclusion criteria:

- Diagnosis of schizophrenia, schizophreniform or schizoaffective disorder as defined by DSM-IV on the basis of the Mini International Neuropsychiatric Interview Plus (Sheehan et al., 1998). Schizophreniform disorder was assessed through a M.I.N.I. diagnosis of psychosis NOS complemented by a diagnosis of schizophreniform disorder according to DSM-IV criteria.
- First episode of psychosis presented within the past 2 years.
- Age 18-40 years.
- Written informed consent.

Exclusion criteria:

- Currently taking antipsychotic medication, for the OPTiMiSE clinical trial, prior use of antipsychotic medication longer than an episode of two weeks in the previous year and/or six weeks lifetime.
- Patients who are coercively treated at a psychiatric ward (based on a judicial ruling).
- Patients who are represented by a legal guardian or under legal custody.
- MRI contraindications including metal implants and pregnancy.

4.3.2. Healthy Control Recruitment

Healthy controls were obtained through poster and gumtree advertisements. Subjects were provided with written study information, and the study was explained in person, whereupon subjects were invited to give written consent. Subjects were aware that they could withdraw at any time, and that their details were confidential. Subjects agreed to their GP being contacted if medically significant results required further investigation.

Inclusion criteria:

- Age 18-40 years.
- Written informed consent.

Exclusion criteria:

- Diagnosis of a psychiatric disorder.
- Current use of psychopharmacological medication.
- Family history of schizophrenia or bipolar disorder.
- MRI contraindications including metal implants and pregnancy.

4.4. Assessments

4.4.1. Patients

The Positive and Negative Syndrome Scale (PANSS) assessment (Kay et al., 1987), and an MRI scan (see below for protocol) were conducted at baseline (timepoint 1), after 5 weeks of antipsychotic treatment (timepoint 2) and a minimum of 10 weeks after baseline (timepoint 3). At the baseline visit demographic data was collected, at timepoint 3 self-report recreational drug use data was collected.

4.4.2. Healthy controls

Healthy controls underwent an MRI scan at baseline (timepoint 1), after 5 weeks (timepoint 2) and a minimum of 10 weeks after baseline (timepoint 3). At the baseline visit demographic data was collected, and self-report recreational drug use data was collected at all timepoints.

4.5. 1H-MRS protocol

All investigations were performed on a 3 Tesla General Electric (Milwaukee, Wisconsin) magnetic resonance scanner. An initial localiser scan was used to identify the anterior commissure-posterior commissure line (AC-PC) and interhemispheric angle. Structural images were acquired using an axial 2D T2-weighted Fast Spin Echo scan and an axial fast fluid-attenuated inversion recovery (FLAIR) scan. A whole brain 3D coronal IR-SPGR (inversion recovery prepared spoiled gradient echo) scan was obtained to localise 1H-MRS voxels.

1H-MRS spectra were acquired using a PRESS sequence (Point RESolved Spectroscopy), echo time TE=30msec; repetition time TR=3000msec, 96 averages. The standard GE PROton Brain Examination (PROBE) sequence was used to supress water signals via a standardised chemically selective suppression (CHESS) water suppression routine. Auto pre-scans were performed twice before each scan to optimise water suppression and shimming. Spectra were collected in left thalamus, where the ROI has a voxel resolution of 15 (right-left) x 20 (anterior-posterior) x 20mm (superior-inferior), localised from sagittal and coronal localisers where the thalamus is widest with the least CSF contamination, obtaining a water linewidth (full-width half maximum) of 10Hz after shimming. Spectra were also obtained from the anterior cingulate, localised from the midline sagittal localizer, with the centre of the 20 x 20 x 20mm ROI being placed 13mm above the anterior portion of the genu of the corpus callosum, 90º to the anterior commissure-posterior commissure line (AC-PC), and making sure to avoid the corpus callosum, see Figure 20. A water linewidth (FWHM) below 7Hz was obtained after shimming.

After subject scanning, PRESS spectra from a phantom containing standardised concentrations of brain metabolites were obtained as a control for scanner drift or sequence abnormalities.

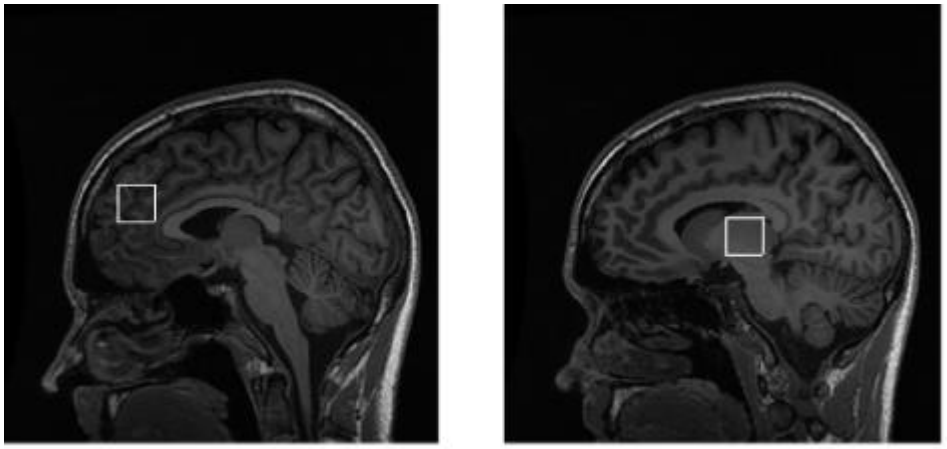

Figure 20 Voxel selection in the anterior cingulate cortex (left image) and thalamus (right image).

4.6. Data analysis

4.6.1. Statistical analysis

All statistical analyses were performed using SPSS version 22 (SPSS inc. Chicago, Illinois, USA). For demographic data, between group differences were assessed using Fisher's Exact Test (2 sided) for categorical variables, and independent samples Student's t-test for continuous variables. Equal variances were assumed unless Levene's test for equality of variances was significant at the 95% confidence level.

CSF-corrected Glx was the primary outcome measure, which measures the combined signals from both glutamate and glutamine, as glutamate and glutamine signals cannot be completely resolved at 3T. Repeated measures ANOVA assessed the effect of time and group on CSF-corrected Glx levels and whether there was a group x time interaction. This was assessed for 1) FEP patients and controls,2) responder and non-responders after 5 weeks of treatment and 3) remission and non-remission patients after 10 months. If significant effects were found, post-hoc tests were conducted using univariate ANOVA. If significant effects were found with Glx, then secondary analyses would examine glutamate. Sphericity (equal variance of differences between groups) was assumed unless Mauchly's Test of Sphericity was violated; in this case the Greenhouse-Geisser correction was used, unless the test statistic (epsilon) was larger than 0.75, in this case the less conservative Huynh-Feldt correction was used (Girden, 1992).

The percentage change in PANSS score between baseline and the 2nd or 3rd timepoint was calculated by first subtracting the minimum score from the PANSS data, as each PANSS item is scored from 1-7 instead of 0-6, before using the following calculation:

((2nd or 3rd timepoint score - baseline score) / baseline score) x 100

The percentage change in Glx and glutamate used the following calculation:

((2nd or 3rd timepoint metabolite value – baseline metabolite value) / baseline metabolite value) x 100

The relationship between the percentage change in PANSS score and the percentage change in Glx was assessed using Spearman bivariate correlations. Outliers were identified using Cook's distance estimates, using the function on SPSS, and excluding values higher than 4/*n*, where *n* equals sample size.

4.6.2. 1H-MRS analysis

LCModel version 6.3-0I (Provencher, 2015, 1993) was used to estimate the concentration of metabolites, by fitting the output to a basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, Gln, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, and taurine), acquired with the same field strength (3 Tesla), localization sequence (PRESS), and echo time (30 msec) (Provencher, 2015). Poorly fitted metabolite peaks (Cramer–Rao minimum variance bounds (CRVB) >20% as reported by LCModel) were excluded from further analysis.

Metabolites are reported as CSF-corrected water-scaled metabolite concentration estimates (institutional units). Cerebrospinal fluid (CSF) contains lower metabolite concentrations and so large volumes of CSF in the ROI would underestimate the metabolite value in the specified brain region. CSF-correction was carried out by segmenting IR-SPGR 3D images into grey, white and CSF volumes using Statistical Parametric Mapping 8, version 6313 (SPM8; Wellcome Department of Imaging Neurosciences, University College London, UK). ROI co-ordinates were obtained from spectra file headers using General Electric's spectroscopy processing tool SAGE, to obtain brain tissue volumes from the same region in the IR-SPGR images. Grey matter and white matter volume in the ROI are added, and this value is used to correct metabolite values using the following calculation:

Uncorrected metabolite x [vol + 1.55 x (1 - vol)] / vol

Vol = grey matter + white matter

The inclusion of “1.55” in the above equation is to correct for the estimated water concentration of the voxel for partial volume CSF contamination, as the spectra are water-scaled (this assumes a CSF water concentration of 55,556 mol/m3 and the LCModel default brain water concentration of 35,880 mol/m3).

4.7. Results: Subject demographics

See Table 6 and Table 7 for subject demographic data. Control and FEP patients did not differ in age, sex or ethnicity. Control subjects had significantly more years of education than FEP patients, and significantly more FEP patients were currently unemployed. At the third timepoint (10 months), control and FEP patients did not differ in their intake of tobacco, alcohol, and recreational drugs.

21 FEP patients were scanned at all three timepoints; antipsychotic-naïve at baseline, after 5 weeks of treatment with antipsychotic medication and at a 3rd timepoint that was a mean of 10 months after the baseline scan. 21 control subjects were scanned at baseline and rescanned 5 weeks later, and 15 of the 21 were scanned at the 3rd timepoint a mean of 9 months after the baseline scan. There were no significant differences in the between-scanning intervals in the two groups (Table 6)

Patients were categorised into medication responders and non-responders after 5 weeks of antipsychotic treatment at the 2nd timepoint, with response defined as a 50% reduction in positive symptoms. After 10 months, at the 3rd timepoint, patients were divided into remission and non-remission groups, with remission defined as a 50% reduction in positive symptoms. There were no significant differences in the number of days between the scanning follow-up timepoints between responder and non-responder groups, and between remission and non-remission groups (Table 6). Remission and non-remission patients did not differ in their intake of tobacco, alcohol, and recreational drugs, with the exception of cannabis, which was used at a higher frequency in non-remission relative to remission patients (Table 7). Patient groups did not differ in their previous exposure to medication at baseline (only three patients had previous antipsychotic use) and duration of medication usage (Table 8).

At baseline no significant differences in PANSS scores were found between responder and non-responder, and remission and non-remission groups. As expected, PANSS scores were significantly higher in the non-responder and non-remission groups at the follow-up time points, although PANSS negative score did not differ between remission and non-remission patients at 10 months (3rd timepoint) (Table 9).

				2nd timepoint (5 weeks)			3rd timepoint (~10 months)		
	Control *n*=15	FEP *n*=21	Patients vs Controls	Non-Responders *n*=9	Responders *n*=12	Non-Responders vs Responders	Non remission *n*=10	Remission *n*=11	Non remission vs Remission
Age, years; mean (SD)	24.5 (4.5)	25.4 (5.3)	T (34)=-0.51; P=0.62	24.2 (5.9)	26.3 (4.8)	T (19)=-0.86; P=0.40	24.3 (5.7)	26.4 (5.0)	T (19)=-.887; P=0.39
Gender, male/female	12/3	15/6	X^2 P=0.71	8/1	7/5	X^2 P=0.18	9/1	6/5	X^2 P=0.15
Education, years; mean (SD)	14.5 (2.7)	12 (2.4)	**T (34)=3.01; P=0.005**	11.9 (2.5)	12.0 (2.4)	T (19)=-0.10; P=0.92	11.5 (2.2)	12.4 (2.5)	T (19)=-.83; P=0.42
Highest level education commenced; University / Professional training / Highschool	7/1/7	5/6/10	X^2 P=0.17	2/2/5	3/4/5	X^2 P=0.86	1/3/6	4/3/4	X^2 P=0.45
Currently employed Y/N	14/1	11/10	**X^2 P=0.011**	3/6	8/4	X^2 P=0.20	4/6	7/4	X^2 P=0.40
Ethnicity (White/Black/Asian/Other)	8/3/2/2	8/8/1/4	X^2 P=0.51	3/2/1/3	5/6/0/1	X^2 P=0.28	3/3/1/3	5/5/0/1	X^2 P=0.44
Days between Baseline and Scan 2; mean (SD) range	37 (14) 27-80	54 (90) 25-444	T (34)=-0.73; P=0.47	38.8 (19.5) 27-89	65.1 (119.5) 25-444	T (19)=-0.65; P=0.52	38 (19) 26-89	69 (125) 25-444	T (19)=-.780; P=0.45
Days between Scan 2 and Scan 3; mean (SD) range	210 (194) 44-604	238 (182) 41-552	T (33)=-0.43; P=0.67	182.8 (144.4) 41-392	279.1 (201.6) 42-552	T (19)=-1.22; P=0.24	215 (170) 42-506	258 (198) 41-552	T (19)=-.533; P=0.60

Table 6 Subject demographics.

Differences between controls and FEP patients, and between non-remission and remission patients, were determined using Student's t test (T) or Fisher's Exact test, 2 tailed (X^2). Significant results P=<0.05 are highlighted in bold.

	Control *n*=15	FEP *n*=21	Patients vs Controls	Non remission *n*=10	Remission *n*=11	Non remission vs Remission
Current smoker: no/yes	6/9	8/13	X^2 P=1.00	2/8	6/5	X^2 P=0.18
Cigarettes/day, mean (SD)	4 (6)	4 (5)	T (34)=-0.07; P=0.95	5 (5)	4 (5)	T (19)=0.599; P=0.56
Current alcohol drinker: no/yes	3/12	9/12	X^2 P=0.28	4/6	5/6	X^2 P=1.0
Alcohol units/week, mean (SD)	5.9 (7.6)	3.1 (4.7)	T (34)=1.35; P=0.19	2.6 (3.3)	3.5 (5.8)	T (19)=-0.45; P=0.66
Cannabis, frequency of use: 0/monthly/weekly/3 times per week/daily	8/0/2/3/2	13/1/0/5/2	X^2 P=0.43	4/0/0/5/1	9/1/0/0/1	**X^2 P=0.03**
Amphetamine, frequency of use: 0/monthly/2xmonth/weekly	15/0/0	21/0/0	NS	10/0/0/0	11/0/0/0	NS
Cocaine, frequency of use: 0/monthly/2xmonth/weekly	12/1/1/1/0	17/3/1/0/0	X^2 P=0.59	9/0/1/0	8/3/0/0	X^2 P=0.21
Ecstasy/MDMA, frequency of use: 0/monthly/2xmonth/weekly	13/0/2/0	20/1/0/0	X^2 P=0.17	10/0/0/0	10/1/0/0	X^2 P=1.0
Ketamine, frequency of use: 0/monthly/2xmonth/weekly	14/0/1/0	20/1/0/0	X^2 P=0.35	10/0/0/0	10/1/0/0	X^2 P=1.0

Table 7 Recreational drug use.
Differences between controls and FEP patients, and between non-remission and remission patients, were determined using Student's t test (T) or Fisher's Exact test, 2 tailed (X^2). Significant results P=<0.05 are highlighted in bold.

			2nd timepoint (5 weeks)			3rd timepoint (10 months)		
Timepoint	Medication information	FEP *n*=21	Non-Responders *n*=9	Responders *n*=12	Responders vs Non-Responders	Non remission *n*=10	Remission *n*=11	Non remission vs Remission
Baseline	More than 2 weeks previous antipsychotic medication use: Yes/No	3/18	1/8	2/10	X^2 P=1.00	1/9	2/9	X^2 P=1.00
	Duration of current treatment at baseline: mean days (SD) range	10 (9) 0-40	11 (12) 0-40	9 (7) 0-17	T (19)=0.49, P=0.63	9 (12) 0-40	11 (6) 0-17	T (19)=-0.58, P=0.57
Baseline – 5 weeks	Duration of treatment; mean days (SD) range	32 (14) 10-80	32 (6) 22-43	32 (18) 10-80	T (19)=-0.06, P=0.95	30 (7) 16-43	34 (18) 10-80	T (19)=-0.64, P=0.53
	% days on medication	87 (26) 18-100	91 (21) 36-100	84 (30) 18-100	T (19)=0.55, P=0.59	87 (23) 36-100	87 (30) 18-100	T (19)=0.06, P=0.96
	Only used Amisulpride: Yes/No	14/7	5/4	9/3	X^2 P=0.40	6/4	8/3	X^2 P=0.66
	Antipsychotic medication: Amisulpride only/Amisulpride+another antipsychotic/Aripiprazole+Risperidone/Aripiprazole	14/4/2/1	5/2/1/1	9/2/1/0	X^2 P=0.78	6/2/1/1	8/2/1/0	X^2 P=0.88
5 weeks – 10 months	Duration of treatment; mean days (SD) range					114 (144) 3-457	199 (179) 32-552	T (19)=-1.186, P=0.25
	% days on medication					62 (38) 10-100	85 (29) 11-100	T (19)=-1.629, P=0.12
	Antipsychotic medication: Amisulpride only/Amisulpride+another antipsychotic/Olanzapine/Aripiprazole/Risperidone/None					3/1/3/1/0/2	5/1/2/1/1/1	X^2 P=0.93
Baseline – 10 months	Duration of treatment; mean (SD) range					161 (145) 36-483	233 (175) 60-580	T (19)=-1.02, P=0.32
	% days on medication					67 (30) 9-100	80 (32) 17-100	T (19)=-0.91, P=0.37
	Antipsychotic medication at 10 months: None/Amisulpride/Olanzapine/Risperidone/Quetiapine/Aripiprazole					5/1/3/0/1/0	3/4/2/1/0/1	X^2 P=0.62

Table 8 Summary of medication information in FEP patients; responder and non-responders at 5 weeks, and remission and non-remission groups at 10 months.
Differences between remission and non-remission patients were determined using Student's t test (T) or Fisher's Exact test (X2) as appropriate. Mean (standard deviation) and range are shown.

		2nd timepoint; 5 weeks			3rd timepoint; 10 months		
PANSS Score	FEP *n*=21	Non-Responders *n*=9	Responders *n*=12	Responders vs Non-Responders	Non remission *n*=10	Remission *n*=11	Non remission vs Remission
Positive	19.6 (4.8)	20.0 (4.9)	19.3 (4.8)	T (19)=0.31; P=0.76	20.0 (4.9)	19.3 (4.9)	T (19)=0.34; P=0.74
Negative	14.5 (5.4)	15.4 (4.6)	13.8 (6.0)	T (19)=0.67; P=0.51	14.7 (4.9)	14.4 (6.0)	T (19)=0.14; P=0.89
General	34.9 (8.8)	37.0 (5.8)	33.3 (10.4)	T (19)=0.97; P=0.35	35.7 (5.5)	34.1 (11.2)	T (19)=0.41; P=0.69
Total	69.0 (16.3)	72.4 (10.1)	66.4 (19.9)	T (19)=0.83; P=0.42	70.4 (10.6)	67.7 (20.7)	T (19)=0.37; P=0.72
Positive	13.3 (5.4)	17.9 (4.6)	9.9 (2.7)	**T (19)=4.99; P=<0.001**			
Negative	13.3 (6.2)	17.4 (6.6)	10.3 (4.6)	**T (19)=2.95; P=0.01**			
General	27.5 (8.8)	35.0 (7.6)	21.9 (4.3)	**T (19)=5.04; P=<0.001**			
Total	54.2 (18.8)	70.3 (16.1)	42.1 (8.9)	**T (19)=5.16; P=<0.001**			
Positive	13.8 (6.1)				18.4 (5.1)	9.5 (2.9)	**T (19)=4.91; P=<0.001**
Negative	11.1 (3.7)				12.0 (4.1)	10.3 (3.4)	T (19)=1.06; P=0.30
General	28.4 (7.4)				33.8 (5.6)	23.5 (5.1)	**T (19)=4.40; P=<0.001**
Total	53.3 (14.6)				64.2 (11.6)	43.4 (9.0)	**T (19)=4.63; P=<0.001**

Table 9 Mean and (standard deviation) of PANSS scores in FEP patients; responder and non-responders at 5 weeks, and remission and non-remission groups at 10 months.
Differences between patient groups were determined using Student's t test (T) or Fisher's Exact Test. P=<0.05 are highlighted in bold.

4.8. Group differences in voxel tissue content

There were no significant differences in ACC voxel tissue content between control and FEP patient groups. In the thalamic voxel, patients had significantly lower grey matter volume (T(40)=2.026, *P*=0.050) but a trend for greater white matter volume than controls at baseline (T(40)=-1.945, *P*=0.059). No differences in voxel tissue content were apparent between patient groups at any timepoint (responder vs non-responder, remission vs non-remission).

4.9. Glutamatergic metabolites in patients with first episode psychosis versus controls.

Anterior cingulate cortex

There were no significant main effects of group (controls vs FEP) (F(1,34)=0.048, *P*=0.828) or time (3 levels; baseline, 5 weeks, 10 months) (F(2,68)=0.558, *P*=0.575) on CSF-corrected Glx in the ACC, and no group by time interaction (F(2,68)=1.255, *P*=0.292).

Thalamus

There were no significant main effects of group (controls vs FEP) (F(1,33)=0.000, *P*=0.993) or time (3 levels) (F(2,66)=1.952, *P*=0.150) on CSF-corrected Glx in the thalamus, and no group by time interaction (F(2,66)=0.013, *P*=0.987).

4.10. Relationship between glutamate metabolites and antipsychotic response after 5 weeks of treatment

4.10.1. Reduction in symptoms over 5 weeks of antipsychotic treatment

Over the first 5 weeks of antipsychotic treatment, symptoms improved in patients as evidenced by an overall reduction in PANSS total, positive and general scores (Table 10). PANSS negative symptoms did not differ between baseline and the 5 week follow-up. One patient did not show an improvement in PANSS positive symptom score, however this patient had poor medication adherence.

Patients were classified into responder and non-responder groups according to the reduction in PANSS positive symptoms, as antipsychotic medication primarily targets this symptom domain. The mean reduction in positive symptoms was 52% (median = -60%); this corresponds to the literature which recommends a 50% cut-off in the reduction of symptoms when assessing treatment response in first episode psychosis patients (Kahn et al., 2008; Leucht et al., 2007). Using a cut-off of 50%, 12 responders and 9 non-responders were identified.

	% Change in symptoms: Baseline - 5 weeks Mean (SD) Range	
PANSS-Positive	-52 (38) -100 to 40	T (20)=5.75; P=<0.001
PANSS-Negative	-10 (87) -100 to 300	T (20)=1.13; P=0.27
PANSS-General	-39 (48) -88 to 112	T (20)=3.29; P=0.004
PANSS-Total	-40 (45) -93 to 103	T (20)=3.71; P=0.001

Table 10 Percentage change in PANSS symptom scores over 5 weeks of treatment with antipsychotic medication.
Differences between PANSS scores at baseline and 5 weeks were determined using Student's t test (T)

4.10.2. Glx levels in responders and non-responders to 5 weeks of antipsychotic treatment

ACC

There was no significant difference between responders and non-responders ($F(1,19)=0.381$, $P=0.545$), no significant effect of time ($F(1,19)=0.012$, $P=0.914$) or group x time interaction ($F(1,19)=1.657$, $P=0.213$) for CSF-corrected Glx levels over the first 5 weeks of treatment, see Figure 23 for mean metabolite values.

Thalamus

There was no main effect of group ($F(1,19)=1.945$, $P=0.179$) or time ($F(1,19)=2.735$, $P=0.115$) for CSF-corrected Glx, but there was a significant group x time interaction ($F(1,19)=6.007$, $P=0.024$) see Figure 21. In the responder group, there was a significant effect of time ($F(1,11)=23.149$, $P=0.001$), reflecting a decrease in Glx between baseline and 5 weeks. There was no significant effect of time in the non-responder group ($F(1,8)=0.155$, $P=0.704$). At baseline, Glx levels in responders and non-responders did not differ ($F(1,19)=0.008$, $P=0.930$), but after 5 weeks of treatment, Glx concentrations were significantly lower in responders than non-responders ($F(1,19)=7.497$, $P=0.013$), see Figure 23 for mean metabolite values.

For Cr-scaled Glx the same results were found; there was no main effect of group ($F(1,19)=1.592$, $P=0.222$) or time ($F(1,19)=2.006$, $P=0.173$), but there was a significant group x time interaction ($F(1,19)=4.959$, $P=0.038$). In the responder group, there was a significant effect of time ($F(1,11)=10.572$, $P=0.008$), reflecting a decrease in Glx/Cr between baseline and 5 weeks. There was no significant effect of time in the non-responder group ($F(1,8)=0.210$, $P=0.659$). At baseline, Glx/Cr levels in responders and non-responders did not differ ($F(1,19)=0.009$, $P=0.924$), but after 5 weeks of treatment, Glx/Cr concentrations were significantly lower in responders than non-responders ($F(1,19)=5.111$, $P=0.036$).

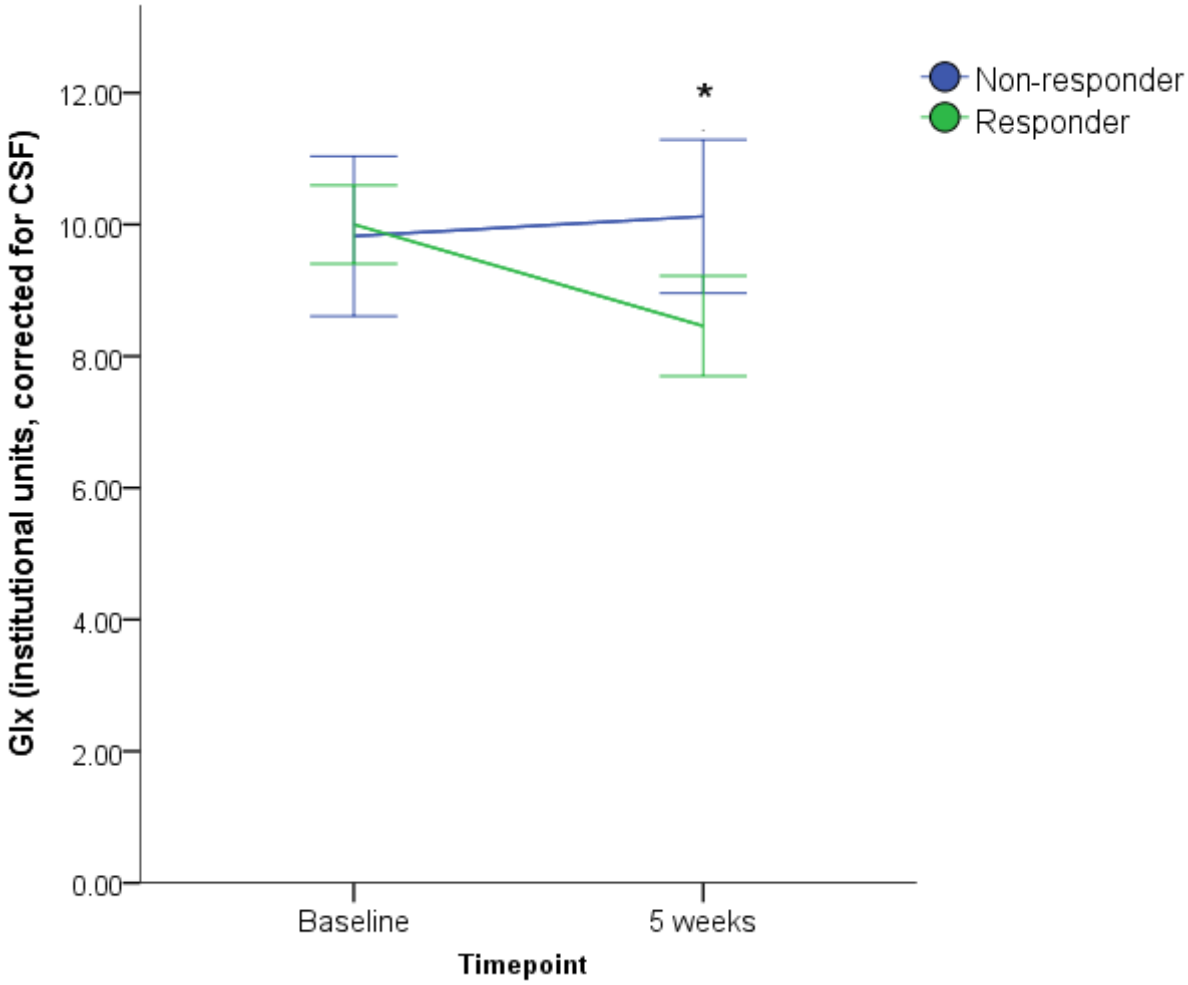

Figure 21 Mean CSF-corrected Glx (institutional units) in the left thalamus, in medication responder and non-responder patient groups at baseline and after 5 weeks of treatment with antipsychotic medication.
Error bars represent the standard deviation. A significant interaction between group and time was found (*P*=0.024). * denotes a significant group difference in the post-hoc analysis (*P*=0.013).

As a significant interaction was found for Glx, associations for glutamate were also investigated. For CSF-corrected glutamate levels, there was a significant effect of time in both groups, with higher glutamate at baseline compared to the 5 week timepoint ($F(1,19)=10.283$, $P=0.005$) but there was no effect of group ($F(1,19)=0.936$, $P=0.345$), and no group x time interaction ($F(1,19)=1.270$, $P=0.274$), see Figure 23 for mean metabolite values.

4.10.3. Controlling for medication adherence

The original analysis was repeated after excluding patients whose self-reported adherence to medication was less than 50% of the time (n=5). Medication adherence was measured as the percentage number of days of medication use from baseline to the 5 week timepoint. Dates of medication use were acquired through patient self-report, corroborated by electronic patient notes. 10 responders and 7 non-responders were investigated in the following analysis.

ACC

There was no effect of group ($F(1,15)=0.795$, $P=0.387$), time ($F(1,15)=0.005$, $P=0.946$) or a group x time interaction ($F(1,15)=2.581$, $P=0.129$) for CSF-corrected Glx.

Thalamus

For CSF-corrected Glx, there was a trend for an effect of time ($F(1,15)=4.123$, $P=0.060$) and a trend for a group x time interaction ($F(1,15)= 3.466$, $P=0.082$). There was no effect of group ($F(1,15)=2.971$, $P=0.105$), see Figure 22.

For CSF-corrected glutamate levels, there was a significant effect of time, with higher glutamate concentrations at the 1st timepoint compared to the 2nd in both groups ($F(1,15)=8.863$, $P=0.009$). There was no effect of group ($F(1,15)=0.430$, $P=0.522$) and no group x time interaction ($F(1,15)=0.275$, $P=0.608$).

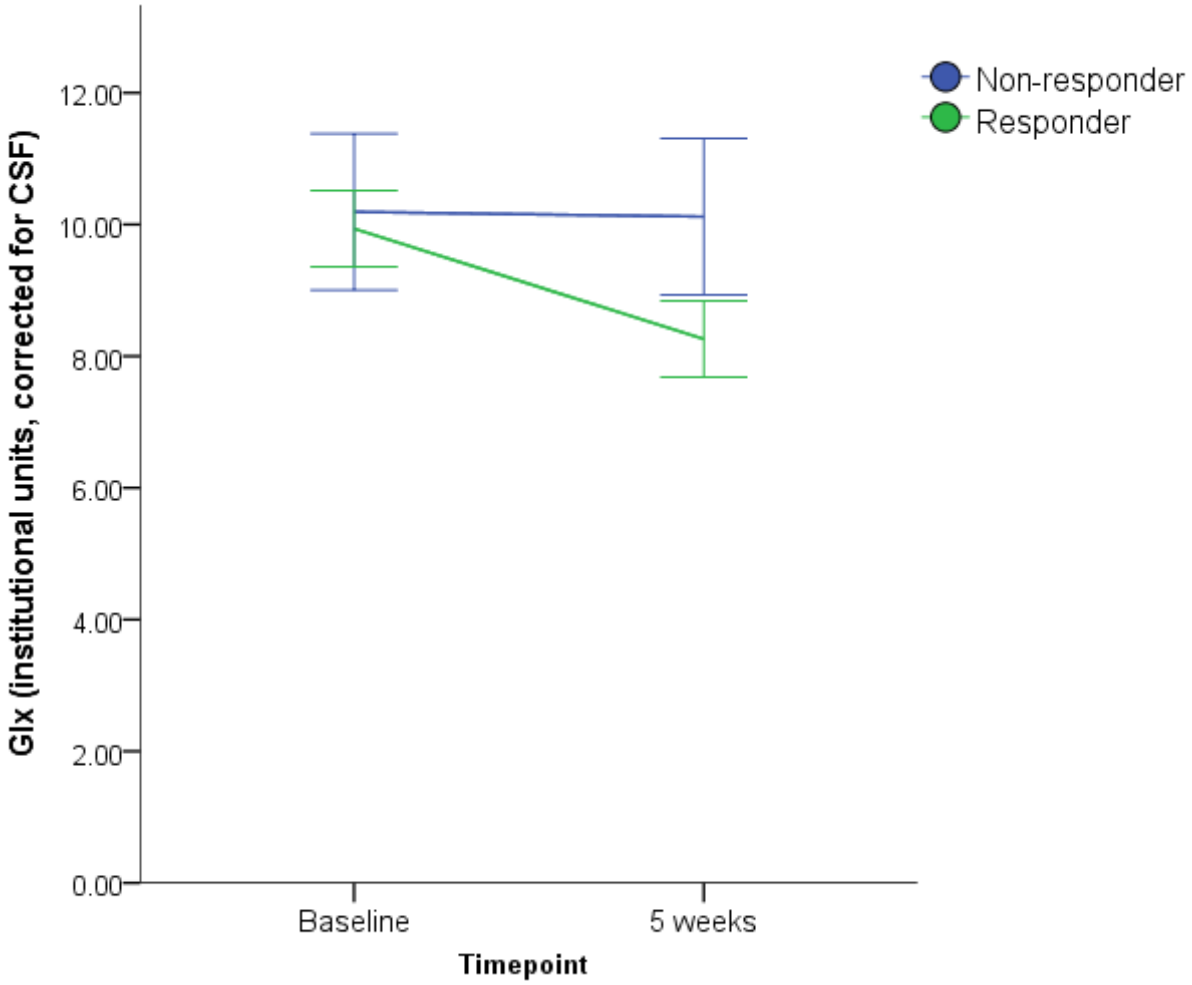

Figure 22 Mean CSF-corrected Glx (institutional units) in the left thalamus, in medication responder and non-responder patient groups at baseline and after 5 weeks of treatment with antipsychotic medication, excluding 4 patients taking medications less than 50% of the time.

Error bars represent the standard deviation. There was a trend for an effect of time (*P*=0.060) and a group x time interaction (*P*=0.082).

4.10.4. Summary of findings: Glx levels in responders and non-responders to 5 weeks of antipsychotic treatment.

Glx levels in the thalamus were reduced after 5 weeks of antipsychotic medication in responders, but not in non-responders. When the analysis was restricted to medication-adherent patients (10 responders and 7 non-responders), the same pattern of results were evident despite the loss of power, with the interaction between time and group for Glx in the thalamus persisting at trend level. The latter is consistent with an effect of antipsychotic treatment on Glx levels in responders. There was no indication that regional Glx or glutamate levels at baseline differed in patients who would or would not subsequently respond to treatment.

	Baseline			5 weeks		
	Responder *n*=12	Non-Responder *n*=9	Statistic	Responder *n*=12	Non-Responder *n*=9	Statistic
ACC						
Glx	17.22 (2.45)	18.90 (3.59)	T (19)=1.278; P=0.22	18.37 (2.60)	17.94 (3.32)	T (19)=-0.337; P=0.74
Glutamate	12.24 (1.49)	13.03 (1.52)	T (19)=1.193; P=0.25	12.51 (1.96)	13.21 (1.53)	T (19)=0.886; P=0.39
Left thalamus						
Glx	9.95 (0.97)	9.89 (2.48)	T (19)=-0.088; P=0.93	8.41 (1.20)	10.18 (1.77)	**T (19)=2.738; P=0.01**
Glutamate	7.69 (0.95)	7.81 (1.43)	T (19)=0.229; P=0.82	6.62 (0.94)	7.29 (1.04)	T (19)=1.551; P=0.14

Figure 23 Glx and glutamate concentrations, Mean (SD), in the anterior cingulate cortex and thalamus in treatment responders and treatment non-responders.
Student's t test (T) statistics are shown, with significant results P=<0.05 highlighted in bold.

4.11. Relationship between remission status at 10 months and brain Glx levels

4.11.1. Reduction in symptoms over 10 months

Between baseline and the 10 month follow up, symptoms improved in patients as evidenced by an overall reduction in PANSS total, positive, negative and general scores (Table 11).

Patients were classified into remission and non-remission groups according to a 50% reduction in PANSS positive symptoms. The mean reduction in positive symptoms was 41%, (median = -53%). 11 remission and 10 non-remission patients were identified.

	% Change in symptoms: Baseline - 10 months Mean (SD) Range	
PANSS-Positive	-41 (78) -100 to 267	T (20)=4.04; P=0.001
PANSS-Negative	-25 (80) -100 to 200	T (20)=2.85; P=0.010
PANSS-General	-30 (43) -100 to 65	T (20)=3.11; P=0.006
PANSS-Total	-38 (43) -100 to 96	T (20)=4.01; P=0.001

Table 11 Percentage change in PANSS symptom scores over 10 months since first presentation with psychotic symptoms.
Differences between PANSS scores at baseline and 10 months were determined using Student's t test (T)

4.11.2. Glx levels in remission and non-remission patients at 10 months

ACC

There were no significant main effects of group (remission vs non-remission) ($F(1,19)=0.547$, $P=0.469$), or time (2 levels; baseline and ~10 months) ($F(1,19)=0.125$, $P=0.727$) on CSF-corrected Glx in the ACC, and no group x time interactions ($F(1,19)=0.000$, $P=0.984$), see Figure 26 for mean metabolite values.

Thalamus

There was no significant main effect of group ($F(1,19)=0.002$, $P=0.962$) or time ($F(1,19)=0.194$, $P=0.665$) on CSF-corrected Glx, but there was a significant group x time interaction ($F(1,19)=11.610$, $P=0.003$), see Figure 24. In the non-remission group, there was a significant effect of time ($F(1,9)=6.701$, $P=0.029$), reflecting an increase in Glx between baseline and ~10 months. There was a strong trend for an effect of time in the remission group ($F(1,10)=4.860$, $P=0.052$), reflecting a decrease in Glx between baseline and ~10 months. Glx levels did not differ between groups at baseline ($F(1,19)=3.313$, $P=0.085$), but

showed a trend for higher Glx in the non-remission than the remission group at ~10 months ($F(1,19)=3.436$, $P=0.079$), see Figure 26 for mean metabolite values.

For Cr-scaled Glx the same results were found; there was no significant main effect of group ($F(1,19)=0.010$, $P=0.920$) or time ($F(1,19)=0.239$, $P=0.630$), but there was a significant group x time interaction ($F(1,19)=6.423$, $P=0.020$). In the non-remission group, there was no longer a significant effect of time ($F(1,9)=2.634$, $P=0.139$). There was now a significant effect of time in the remission group ($F(1,10)=5.370$, $P=0.043$), reflecting a decrease in Glx/Cr between baseline and ~10 months. Glx/Cr levels did not differ between groups at baseline ($F(1,19)=2.995$, $P=0.102$), and no longer significantly differed at ~10 months ($F(1,19)=1.359$, $P=0.258$).

As a significant interaction was found for Glx, associations for glutamate were also investigated. There were no significant main effects of group ($F(1,19)=0.136$, $P=0.717$), or time ($F(1,19)=0.234$, $P=0.634$) on CSF-corrected glutamate, and no group x time interactions ($F(1,19)=2.293$, $P=0.146$), see Figure 26 for mean metabolite values.

As the frequency of cannabis use significantly differed between remission and non-remission groups at 10 months (see Table 7 above), analyses were repeated with cannabis frequency entered as a co-variate. For Glx in the ACC and for glutamate in the thalamus no significant effects were found. For Glx in the thalamus, there was no significant main effect of group ($F(1,18)=0.028$, $P=0.869$) or time ($F(1,18)=0.172$, $P=0.683$) but there was a significant group x time interaction ($F(1,18)=6.273$, $P=0.022$). In the non-remission group, there was no longer a significant effect of time ($F(1,8)=1.554$, $P=0.248$). There was now a significant effect of time in the remission group ($F(1,9)=6.970$, $P=0.027$), reflecting a decrease in Glx between baseline and ~10 months. Glx levels did not differ between groups at baseline ($F(1,18)=1.443$, $P=0.245$), and no longer significantly differed at ~10 months ($F(1,18)=2.046$, $P=0.170$).

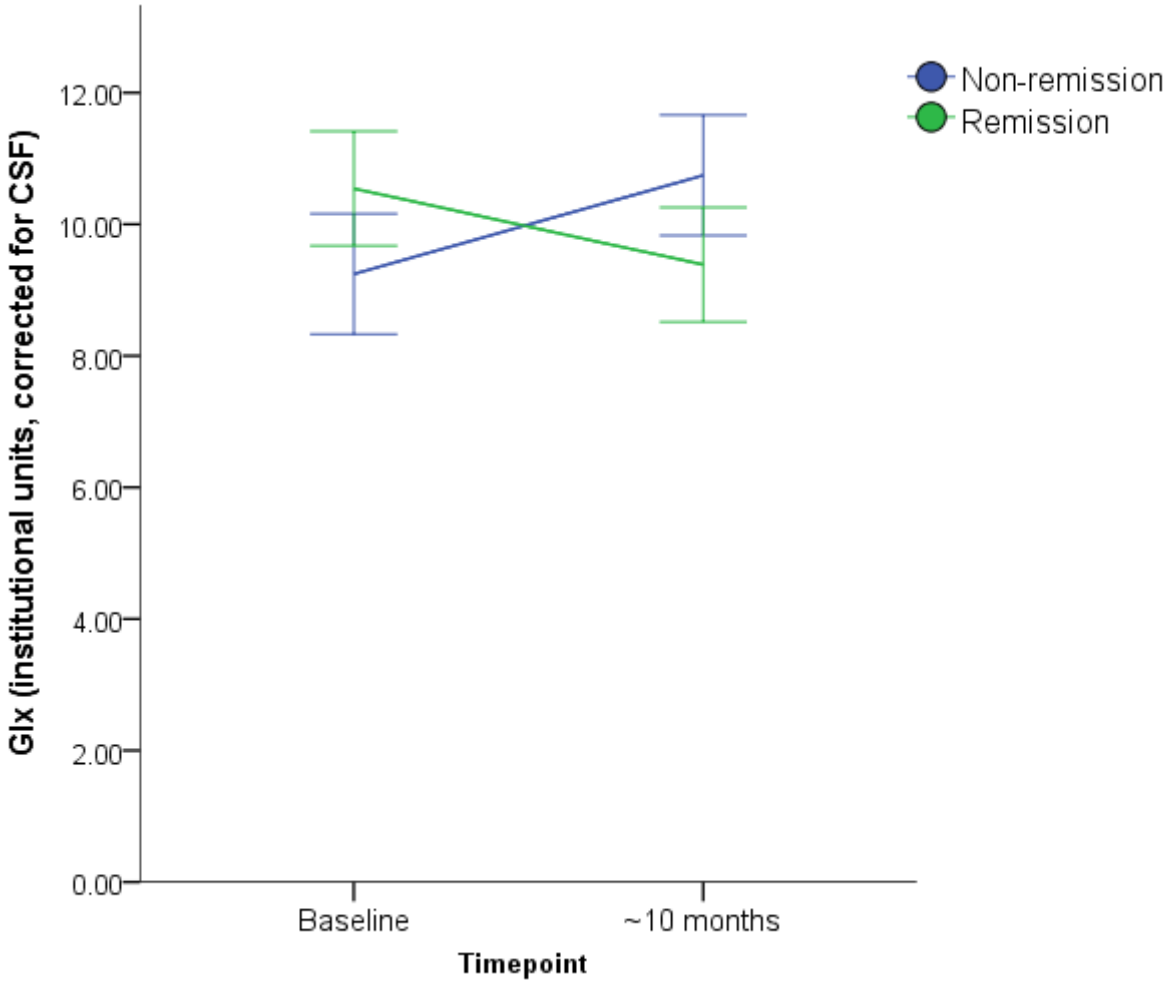

Figure 24 Mean CSF-corrected Glx (institutional units) in the left thalamus at baseline and 10 months, in remission and non-remission groups.

Error bars represent the standard deviation. There was a significant group x time interaction (*P*=0.003), and a trend for higher Glx in the non-remission group at ~10 months (*P*=0.079).

4.11.3. Controlling for medication adherence

The original analysis was repeated after excluding patients whose self-reported adherence to medication was less than 50% of the time (n=6). Medication adherence was measured as the percentage number of days of medication use from the 1st to the 3rd timepoint. Dates of medication use were acquired through patient self-report, corroborated by electronic patient notes. 9 remission and 6 non-remission were investigated in the following analysis.

ACC

There were no significant main effects of group (remission vs non-remission) ($F(1,13)=0.538$, $P=0.476$), or time (2 levels; baseline and ~10 months) ($F(1,13)=0.001$, $P=0.981$) on CSF-corrected Glx in the ACC, and no group x time interactions ($F(1,13)=1.097$, $P=0.314$).

Thalamus

There was no significant main effects of group ($F(1,13)=0.223$, $P=0.645$) or time ($F(1,13)=0.033$, $P=0.859$) on CSF-corrected Glx, but there was a significant interaction between group and time ($F(1,13)=9.377$, $P=0.009$), see Figure 25. Post-hoc tests revealed a significant effect of time in the remission group ($F(1,8)=9.152$, $P=0.016$), reflecting a decrease in Glx between the baseline and ~10 months. In the non-remission group, there was no significant effect of time ($F(1,5)=2.384$, $P=0.183$). Glx levels did not differ between groups at baseline ($F(1,13)=1.161$, $P=0.301$), but showed a trend for higher Glx in the non-remission than the remission group at ~10 months ($F(1,13)=3.834$, $P=0.072$).

For glutamate, there was no significant main effect of group ($F(1,13)=0.148$, $P=0.706$) or time ($F(1,13)=0.554$, $P=0.470$), but there was a trend for an interaction between group and time (CSF-corrected $F(1,13)=3.393$, $P=0.088$).

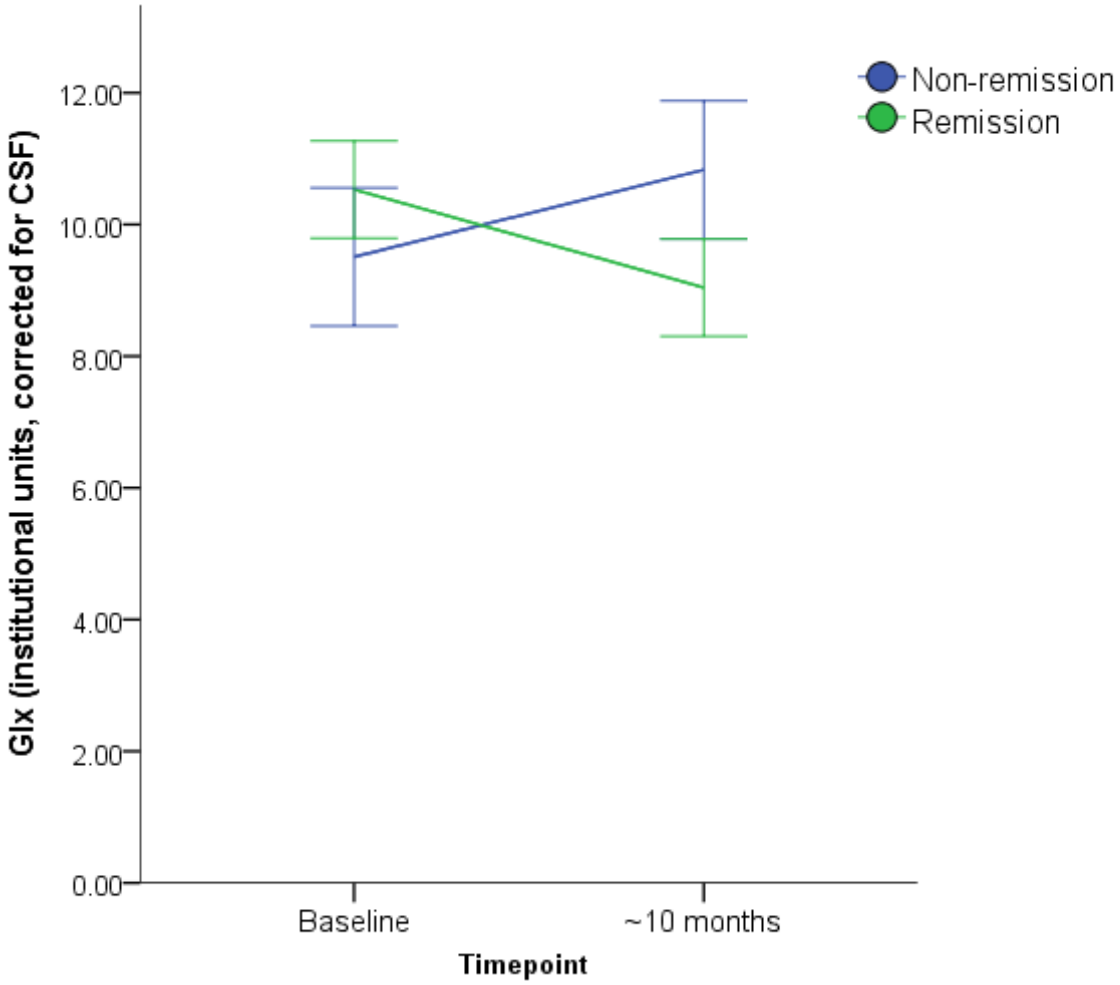

Figure 25 Mean CSF-corrected Glx (institutional units) in the left thalamus at baseline and ~10 months, in remission and non-remission groups, excluding 6 patients taking medications less than 50% of the time.

Error bars represent standard deviation. There was a significant group x time interaction (*P*=0.009), and a trend for higher Glx in the non-remission group at ~10 months (*P*=0.072).

4.11.4. Summary of findings: Glx levels in remission and non-remission at the 10 month follow-up.

For Glx in the thalamus, a significant interaction between remission groups over ~10 months was found, which was driven by a trend for higher Glx concentrations in non-remission patients at the 10 month timepoint relative to remission patients. This finding was not attributable to an effect of variations in adherence to medication. When the analysis was restricted to medication-adherent patients (9 remission and 6 non-remission), the same pattern of results were evident despite the loss of power, with the interaction between time and group for Glx in the thalamus remaining significant. The latter is consistent with an effect of antipsychotic treatment on Glx levels in patients who enter remission. Furthermore, for glutamate, a trend for an interaction between remission groups emerged when the analysis was restricted to medication adherent patients. There was no indication that regional Glx or glutamate levels at baseline differed in patients who would or would not subsequently respond to treatment.

	Baseline			10 months		
	Remission *n*=11	No Remission *n*=10	Statistic	Remission *n*=11	No Remission *n*=10	Statistic
ACC						
Glx	17.59 (2.79)	18.34 (3.38)	T (19)=0.557; P=0.58	17.41 (1.59)	18.14 (2.34)	T (19)=0.843; P=0.41
Glutamate	12.41 (1.66)	12.76 (1.41)	T (19)=0.529; P=0.60	11.65 (1.68)	12.36 (1.80)	T (19)=0.933; P=0.36
Left thalamus						
Glx	10.54 (1.90)	9.24 (1.27)	T (19)=-1.820; P=0.09	9.39 (1.68)	10.74 (1.8)	T (19)=1.854; P=0.08
Glutamate	8.04 (1.37)	7.41 (0.79)	T (19)=-1.269; P=0.22	7.71 (1.34)	8.05 (0.91)	T (19)=0.684; P=0.50

Figure 26 1H-MRS metabolite levels, mean (SD), in the anterior cingulate cortex and thalamus in patients who enter remission, and in patients who do not enter remission at the 10 month follow-up.

Student's t test (T) statistics are shown.

4.12. The relationship between longitudinal changes in Glx levels and longitudinal changes in symptom severity.

4.12.1. Change from baseline to 5 weeks

The association between the percentage change in Glx levels between baseline and 5 weeks with the change in PANSS positive score was assessed, as antipsychotic treatment is proposed to primarily affect this symptom domain.

ACC

The percentage change in Glx levels between baseline and 5 weeks did not correlate with the percentage change in the PANSS positive score (*rho*=-.174, *P*=0.451). Secondary analyses found no significant correlations with the PANSS total score (*rho*=-.130, *P*=0.575) and PANSS negative score (*rho*=.051, *P*=0.827).

Thalamus

There was a positive correlation between the percentage change in Glx levels and the change in PANSS positive score (*rho*=.486, *P*=0.026; Figure 27). Secondary analyses found no significant correlations with the PANSS total or negative score (*rho*=.378, *P*=0.091 and *rho*=.209, *P*=0.364 respectively).

As a significant result for Glx was found, correlations with glutamate were also examined. There was a positive correlation between the percentage change in glutamate levels and the change in PANSS positive score (*rho*=.498, *P*=0.022; Figure 28). Secondary analyses found no significant correlations with the PANSS total or negative score (*rho*=.331, *P*=0.143 and *rho*=.215, *P*=0.350 respectively).

For the significant correlations with PANSS positive score, Cook's distance estimates identified 1 outlier for Glx and 2 outliers for glutamate. When these outliers were excluded, the positive correlation between PANSS positive score and percentage change in Glx and glutamate remained significant (*rho*=.446, *P*=0.049 and *rho*=.526, *P*=0.021 respectively).

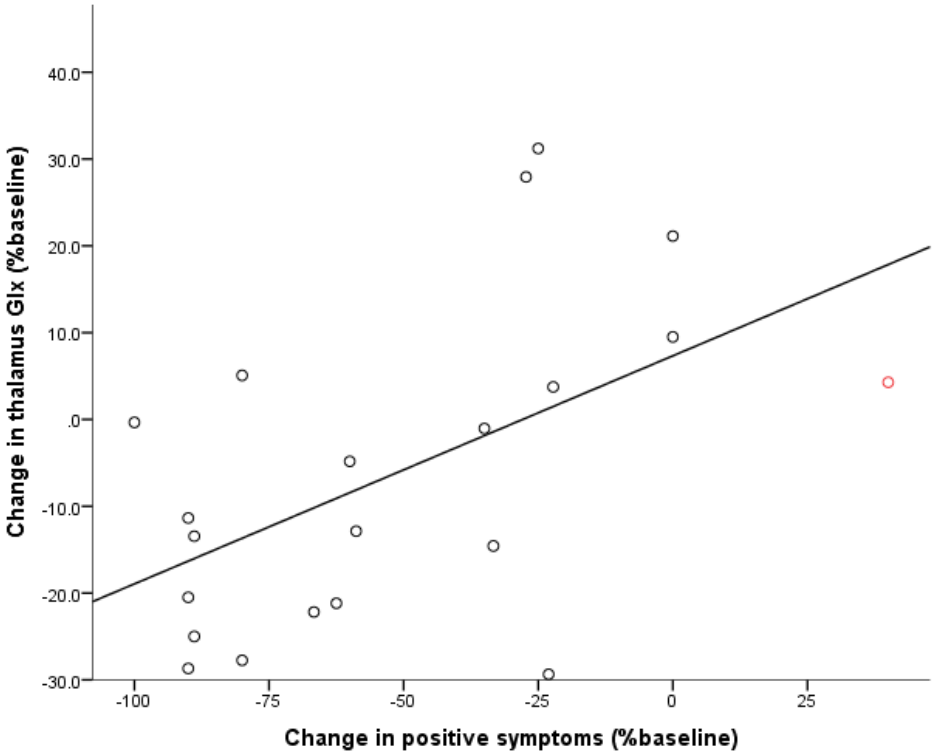

Figure 27 The relationship between the change in thalamic Glx from baseline to 5 weeks (%baseline) and the percentage change in PANSS positive score (*rho*=.486, P=0.026). One outlier is highlighted in red.

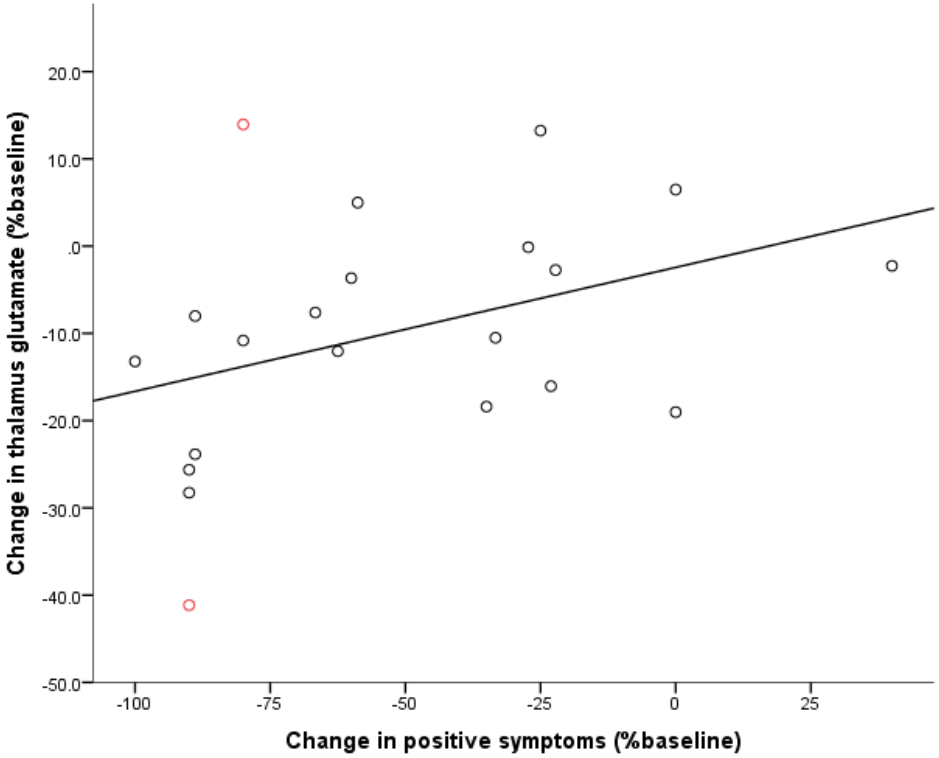

Figure 28 The relationship between the change in thalamic glutamate from baseline to 5 weeks (%baseline) and the percentage change in PANSS positive score (*rho*=.498, *P*=0.022). Two outliers are highlighted in red.

4.12.2. Change from baseline to 10 months

ACC

The percentage change in Glx levels between baseline and 10 months did not correlate with the percentage change in the PANSS positive score (*rho*=.063, *P*=0.786). Secondary analyses found no significant correlations with the PANSS total score and PANSS negative score (*rho*=.134, *P*=0.563 and *rho*=.125, *P*=0.589 respectively).

Thalamus

There was a positive correlation between the percentage change in Glx levels between baseline and 10 months and the percentage change in PANSS positive score (*rho*=.601, *P*=0.004; Figure 29). Secondary analyses found a positive correlation for the PANSS total (*rho*=.528, *P*=0.014; Figure 31) but not the PANSS negative score (*rho*=.161, *P*=0.485).

As a significant result for Glx was found, correlations with glutamate were also examined. The percentage change in glutamate levels in the thalamus between baseline and the 3rd timepoint did not correlate with the change in the PANSS positive score (*rho*=.166, *P*=0.473). Secondary analyses found no significant correlations with the PANSS total or negative score (*rho*=.260, *P*=0.256 and *rho*=.086, *P*=0.710 respectively).

For the significant correlations with PANSS positive score, Cook's distance estimates identified 1 outlier for Glx. When the outlier was excluded, the positive correlation between PANSS positive score remained significant for Glx (*rho*=.552, *P*=0.012), see Figure 30. For significant correlations between the change in Glx and the change in PANSS total score, Cook's distance estimates identified the same outlier. When excluded, the positive correlation with PANSS total score remained significant (*rho*=.473, *P*=0.035).

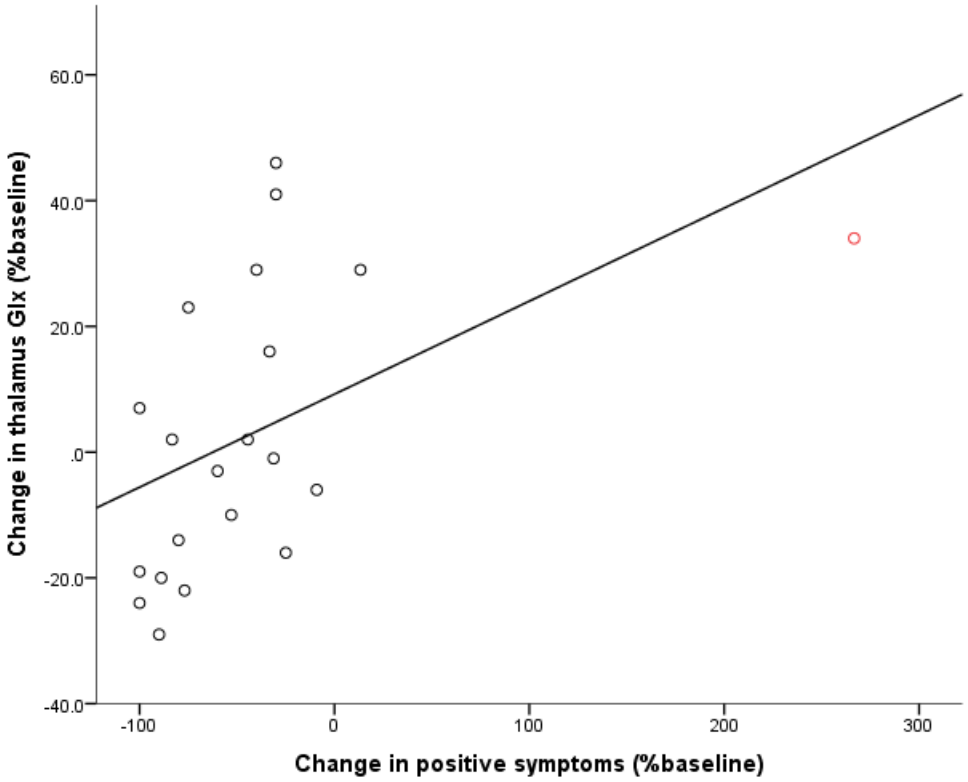

Figure 29 The relationship between the percentage change in thalamic Glx from baseline to ~10 months and the percentage change in PANSS positive score. One outlier is highlighted in red.

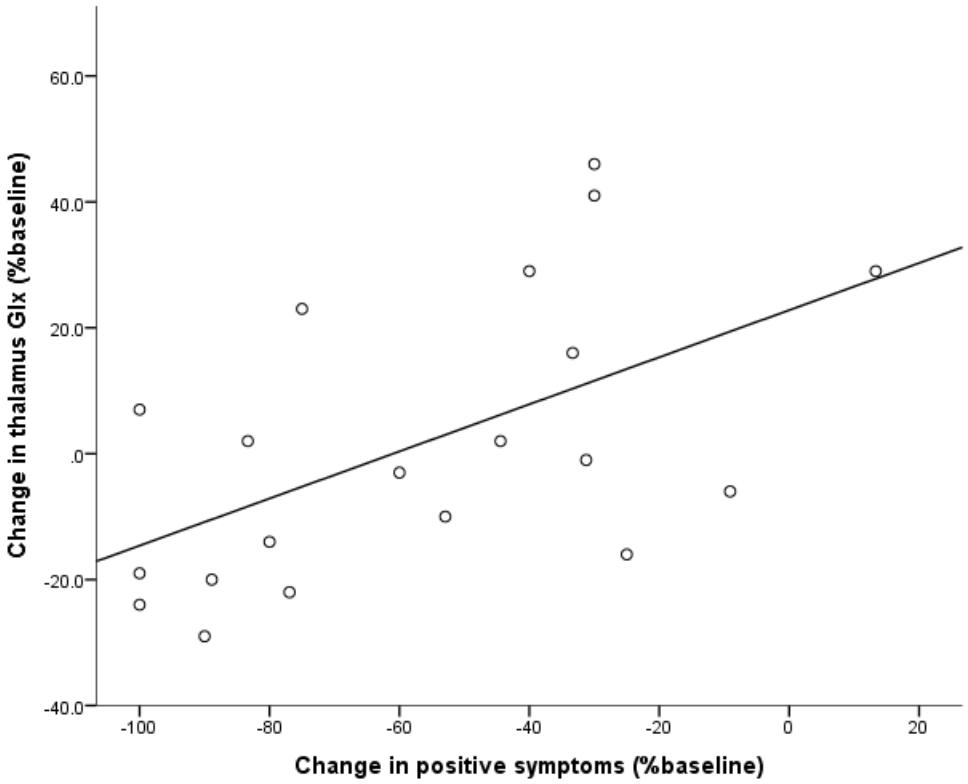

Figure 30 The relationship between the percentage change in thalamic Glx from baseline to ~10 months and the percentage change in PANSS positive score, excluding one outlier labelled in the previous graph.

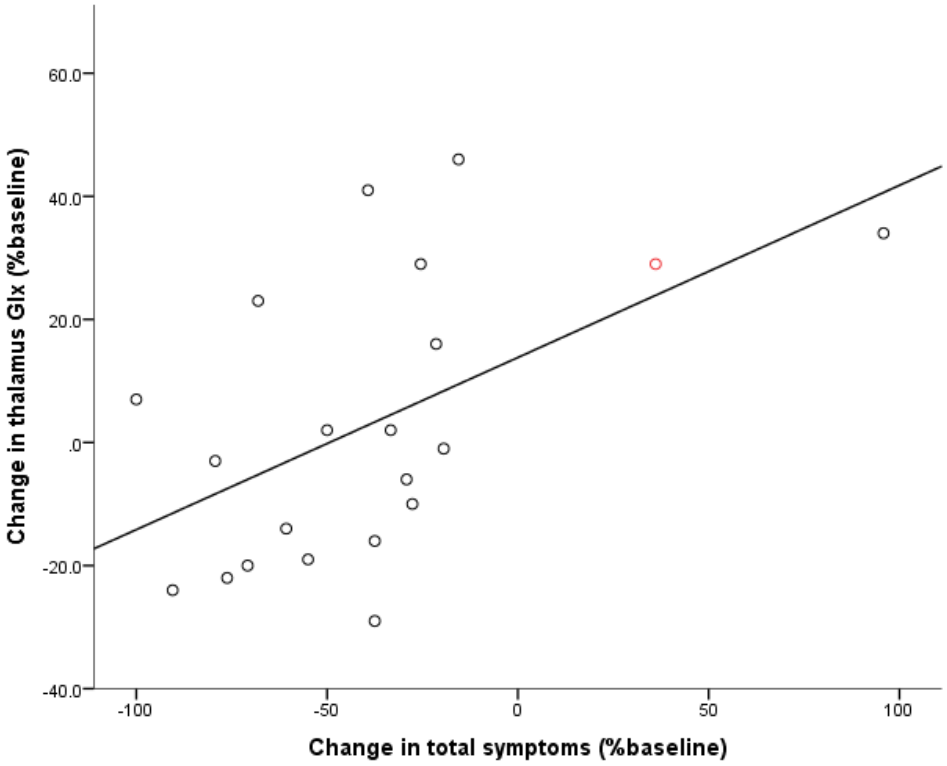

Figure 31 The relationship between the percentage change in thalamic Glx from baseline to ~10 months and the percentag change in PANSS total score. One outlier is highlighted in red.

4.13. Summary of findings

This longitudinal study examined glutamate metabolite changes during antipsychotic treatment in patients with first episode psychosis who had previously received minimal or no treatment. The main finding was that thalamic Glx concentrations decreased over the first 5 weeks of antipsychotic treatment in the subgroup of patients whose symptoms improved, and remained lower at the ~10 month follow-up in the subgroup of patients who were in remission. However, there was no change in thalamic Glx levels in patients who did not respond to treatment. Glutamate/Glx concentrations at baseline did not predict the subsequent response to treatment, and there were minimal differences in Glx and glutamate in the patient group as a whole compared to controls. These findings suggest that clinically effective antipsychotic treatment is associated with a longitudinal reduction in thalamic Glx levels, but that this reduction is not evident in patients whose psychotic symptoms do not improve with treatment.

A limitation of this study is that it is not possible to determine whether antipsychotic medication causes the reduction in thalamic Glx levels, rather than being a natural progression of the illness. Although the findings remained significant at trend level when the analyses were restricted to patients who were adherent to treatment, patient self-reports of medication adherence may not be reliable, and blood monitoring of medication adherence would have been preferable. In order to determine whether antipsychotic medication mediates the reduction in Glx, a group of first-episode psychosis patients not receiving antipsychotic medication would be required. This would present with significant ethical challenges as treatment would be withheld for at least 5 weeks.

The finding of no significant difference in Glx between first episode psychosis patients and healthy controls in the ACC is consistent with the meta-analysis (Chapter 2.2), as no significant differences between FEP patients and controls were detected in the medial frontal cortex. However two studies at 4T found elevated glutamine levels in FEP (Chapter 2.2). Similarly in the thalamus, the present study did not detect differences in Glx between FEP patients and healthy controls. The meta-analysis identified case-control differences in thalamic glutamine levels but not in glutamate or Glx concentrations. As the present study was conducted at 3T it was not possible to resolve glutamine in the ACC or thalamus. In the meta-analysis there were insufficient studies to assess thalamic glutamine levels in first episode patients separately, and so future studies at 4T in this clinical group will help to clarify the nature of glutamatergic abnormalities in both the ACC and thalamus. Significant differences in glutamine, but not glutamate or Glx, may be detected as glutamate release into the synapse is rapidly metabolised

to glutamine, and so glutamine may be a more sensitive marker of neurotransmission turnover (Rothman et al., 2011).

There was large variation in the timing of the third timepoint (~10 months), which may affect the findings as glutamatergic differences vary with the stage of the disorder, as shown in the meta-analysis in Chapter 2.2. However the findings from the 2nd timepoint (at 5 weeks) were largely consistent with those at the 3rd timepoint (~10 months), and there was no significant difference in the time to follow-up between remission and non-remission groups.

Data on recreational drug use was available at baseline and the 2nd timepoint for 15 patients recruited from the OPTiMiSE clinical trial but was not available for the 6 patients recruited from the TreatFEP study. Therefore significant differences in recreational drug use between responder and non-responder groups at baseline and the 2nd timepoint could not be assessed, and if present, may mediate the significant difference in thalamic Glx levels between the patient groups. However data on recreational drug use was available for all patients at the 3rd timepoint, and although significant differences in cannabis use were found between remission and non-remission groups, the significant interaction between group and time for thalamic Glx levels remained when cannabis use was entered as a covariate.

It is of interest that Glx levels in the ACC did not differ between responders and non-responders, and instead differences were only found in the thalamus, contrary to three previous reports of higher levels of glutamate in the ACC of treatment-resistant patients (this will be discussed at greater length in the discussion chapter below, section 5.2.2) (Demjaha et al., 2014; Egerton et al., 2012; Mouchlianitis et al., 2015). This may be due to the low severity of negative symptoms in the present sample, as they are found to correlate with glutamate levels in the ACC (Egerton et al., 2012), consistent with the role of the ACC in modulating affect (Fornito et al., 2009). Furthermore chronic patients rather than first episode psychosis patients were examined in two of the three previous studies examining treatment response (Demjaha et al., 2014; Mouchlianitis et al., 2015). The ACC is connected to cognitive prefrontal and emotional limbic regions, and is proposed to modulate affect.

This study only examined glutamatergic metabolites in the ACC and thalamus. Thalamic Glx levels were negatively correlated with the change in positive symptoms, and were higher in non-responders compared to responders, and in non-remission patients compared to those in remission. Elevated thalamic Glx levels in non-responders may underlie positive symptoms, as the thalamus is an integral gateway in sensory processing (Pergola et al., 2015). The thalamus integrates sensory input with information from a number of higher-order limbic regions via a

series of thalamo-cortical loops, and so abnormalities in this brain region would likely impact upon functioning in other brain areas (please see chapter 5 below for a detailed discussion regarding the possible neuro-circuitry of glutamatergic abnormalities in non-responders, and their relation to the dopamine hypothesis of schizophrenia). Thalamo-cortical projections are glutamatergic, whereas thalamic tone is maintained by GABAergic interneurons (Clinton and Meador-Woodruff, 2004b). Abnormalities in GABA are thought to also play a role in schizophrenia, as elevated glutamate release is proposed to result from disinhibited GABAergic interneurons (Lisman et al., 2008). Future 1H-MRS studies using edited acquisition sequences that are able to measure GABA concentrations in the brain may further elucidate the pathways involved.

The meta-analysis in Chapter 2.2 found that glutamate measures were elevated in the medial temporal lobe, basal ganglia and thalamus of patients. It therefore would be of interest to determine whether Glx levels in the medial temporal lobe and basal ganglia relate to treatment response. The latter is supported by one report of reduced glutamate levels in medication-naïve FEP patients in the striatum following 4 weeks of treatment with risperidone (de la Fuente-Sandoval et al., 2013). Although the patient sample was not subdivided according to response, there was a 30% reduction in PANSS total score in the sample as a whole, suggesting that most patients were treatment responders. In Chapter 2.3, the multicentre analysis found that medial temporal Glx levels correlated with negative symptoms, but no studies to date have investigated its relation to treatment response. It would also be of interest to incorporate measures of cognitive performance in a future study, as glutamatergic abnormalities in the medial temporal lobe are suitably placed to underlie cognitive deficits in schizophrenia.

CHAPTER 5 – Discussion

5. Summary of findings

This thesis examined the use of proton magnetic resonance spectroscopy to measure glutamate function in psychosis. In Chapter 2, a meta-analysis of 1H-MRS studies indicated that schizophrenia is associated with elevations of glutamatergic metabolites in the medial temporal cortex, the thalamus, and the basal ganglia (section 2.2). There was also some evidence that alterations in glutamate metabolite levels varied according to clinical stage: elevated medial frontal levels were evident in high risk and FEP patients but not in chronic schizophrenia; conversely, medial temporal Glx levels were elevated in chronic patients but were not altered in high risk or FEP groups. Overall, a systematic review (section 2.1) and a multicentre analysis (section 2.3) did not find robust associations between glutamate measures and symptom scores, although these analyses could not assess whether longitudinal changes in glutamate levels were related to changes in symptom severity over time. The effect of medication on glutamate measures was less clear; glutamate measures were not related to CPZ dose equivalents in the meta-analysis, but a more sensitive analysis of multicentre data revealed a trend for a negative correlation with medial frontal glutamate (section 2.3). Chapter's 1 and 2 concluded that, although glutamate alterations are present in schizophrenia, and have been found to differentiate patients based on their clinical response, it is still unclear whether glutamate levels predict the therapeutic response or whether effective treatment is associated with a reduction in glutamate. To address this question, raised in the meta-analysis and multicentre data analysis, a longitudinal 1H-MRS study of antipsychotic response in first episode psychosis was conducted. Firstly, to examine the feasibility and determine the sample size for this study, in Chapter 3 the test-retest reproducibility and reliability of longitudinal 1H-MRS glutamate measures was determined. Chapter 4 outlined the methodology and the results of a 1H-MRS study that aimed to examine the relationship between 1H-MRS glutamate measures and the acute and medium-term response to antipsychotic medication in first episode schizophrenia.

5.1.1. Initial response to treatment

The main finding of the 1H-MRS study of antipsychotic response in first episode psychosis (Chapter 4) was that longitudinal changes in Glx levels in the thalamus were related to the therapeutic effectiveness of antipsychotics. Prior to treatment, thalamic Glx levels were similar in patients who subsequently responded to medication and in those who did not

respond. However, after 5 weeks of antipsychotic treatment, Glx levels were lower than at baseline in responders, but not in non-responders. These findings remained significant at trend level when analysis was limited to only medication adherent patients, suggesting that the changes in thalamic Glx were related to the degree of response to the antipsychotic medication, as opposed to the natural course of the illness or between group differences in medication-adherence.

In accordance with the reduction in thalamic Glx in the treatment responder group, a direct correlation between the longitudinal reduction in PANSS positive score over the first 5 weeks of treatment and the reductions in both thalamic glutamate and thalamic Glx levels over the same time period was observed. These correlations were specific to positive psychotic symptoms: there were no correlations with the change in either negative symptoms or the total PANSS score. As the main effect of antipsychotic medication is on positive symptoms, this is also in line with an effect of treatment.

5.1.2. Remission after 10 months

Consistent with the observations over five weeks of treatment, the second major finding was that patients who were in remission after 10 months of treatment had lower thalamic Glx levels than patients who were not in remission. Over 10 months, thalamic Glx levels reduced in remitted patients, but increased in patients who were not in remission. Furthermore, the reduction in thalamic Glx levels over 10 months correlated with the reduction in PANSS total score over the same period, which was again driven by changes in the positive symptom domain.

Although it is not possible to determine whether the longitudinal change in thalamic Glx in responders was an effect of antipsychotic treatment specifically, as opposed to the natural course of the illness, the fact that the finding remained significant when the analysis was restricted to patients who were adherent to treatment supports this interpretation. Furthermore, a trend for longitudinal thalamic *glutamate* changes to relate to remission status only emerged when the analysis was restricted to medication adherent patients, implying that glutamate levels are also related to the therapeutic effectiveness of antipsychotics. The change in Glx was also not attributable to group differences in cannabis misuse, as the interaction remained when the frequency of cannabis use was entered as a covariate.

5.1.3. Summary of Glx findings relating to clinical status

Taken together, the data suggest that at both 5 weeks and at 10 months, longitudinal changes in thalamic Glx levels were related to the clinical status of the patients at follow up. At both timepoints, reductions in Glx levels from baseline were associated with a good clinical response and / or remission. On the other hand there was no reduction, or an increase in Glx levels in the patients who had a relatively poor outcome. In addition, across all patients, the improvement in positive symptoms following treatment for both 5 weeks and 10 months was directly correlated with the longitudinal reductions in thalamic Glx levels. These findings indicate that in patients with a schizophreniform psychosis, a good response to the initial treatment with antipsychotic medication is associated with longitudinal reductions in thalamic Glx levels.

5.2. Comparison with previous studies

5.2.1. Previous studies of glutamate in first episode psychosis

Previous studies of antipsychotic naïve/minimally treated FEP have reported higher ACC glutamine levels than in controls (Bartha et al., 1997; Bustillo et al., 2010; Theberge et al., 2002), which was also detected in my meta-analysis (Chapter 2.2). Longitudinal studies suggest that this elevation in glutamine levels is still evident after 6 - 10 months of antipsychotic treatment (Bustillo et al., 2010; Theberge et al., 2002). The present longitudinal 1H-MRS study at 3T was unable to evaluate glutamine specifically. However, in both the meta-analysis (Chapter 2.2) and my present study I did not detect differences in ACC Glx concentrations between FEP patients and controls.

Similarly, I did not detect differences in Glx between patients and controls in the thalamus. Thalamic glutamate function has not been extensively studied in FEP patients, but most studies have not found differences in glutamate or Glx levels between FEP patients and controls (Bustillo et al., 2010; Galińska et al., 2009; Szulc et al., 2004; Theberge et al., 2002). One previous study reported that thalamic glutamine levels were elevated in antipsychotic-naïve FEP patients (Theberge et al., 2002), and that these were reduced after 30 months of treatment (Theberge et al., 2007). This is consistent with the meta-analysis finding of elevated thalamic glutamine in cases, although the meta-analysis was unable to assess FEP patients separately (Chapter 2.2).

One potential factor that may have contributed to the absence of differences in 1H-MRS measures at baseline in the present study is that the control group was relatively small (n=15), but nonetheless comparable with other studies. I cannot therefore exclude the

possibility that true differences were not detected because of a lack of statistical power, although the absence of significant differences in ACC and thalamic Glx levels is consistent with the meta-analysis (Chapter 2.2). The modest size of the control group reflects the fact that the study was designed to compare glutamate measures in responders and non-responders to treatment *within* the patient sample, rather than in patients with schizophrenia and controls (see the power analysis in Chapter 3). A further consideration with respect to measures of glutamine is that in the present study, which was conducted using a 3T scanner, it was not possible to assess glutamine in every subject, due to a poor signal to noise ratio.

Notwithstanding the lack of case-control differences, the data from the present study clearly suggest that in the first episode of psychosis, glutamate dysfunction is more evident in patients in whom antipsychotic treatment is relatively ineffective than in the patient sample as a whole. The absence of significant longitudinal differences in 1H-MRS measures within the control sample indicates that these were stable over time, and did not vary for technical or other non-specific reasons. This is consistent with the test-retest analysis, which showed a good reproducibility and reliability of glutamate measures in healthy controls over an equivalent time-period (Chapter 3). This is important, as it could otherwise have been a confounding factor in the interpretation of the longitudinal changes in the patient sample.

5.2.2. Previous studies of glutamate measures relating to clinical status

No studies have previously assessed whether ACC or thalamus glutamate levels in unmedicated FEP patients predict the subsequent response to antipsychotic treatment. One cross-sectional study has compared glutamate function in the thalamus and ACC in patients with first episode psychosis who were or were not in remission following treatment (Egerton et al., 2012), and two cross-sectional studies examined ACC glutamate levels in chronic patients after they had been defined as treatment resistant on the basis of previous treatments (Demjaha et al., 2014; Mouchlianitis et al., 2015). The former study found higher ACC glutamate levels in patients who were not in remission following treatment, relative to those in remission (Egerton et al., 2012), with no group difference in the thalamus. The latter studies found higher ACC glutamate levels in patients who had failed to respond to previous treatment with at least two antipsychotic drugs, relative to those in remission (Mouchlianitis et al., 2015) and to healthy controls (Demjaha et al., 2014) (also with no difference in the thalamus). A key issue in the interpretation of these previous studies was that it was not possible to determine whether the group differences

predated antipsychotic treatment or were secondary to this. My findings suggest that differences in glutamate metabolites between responders and non-responders are not present prior to treatment, but emerge after it has been delivered.

The patients in the Egerton et al., study were examined on average 10 months after first presentation, which is similar to the ~10 month timepoint in the present study. However, the present study detected a difference in the thalamus but not the ACC at 10 months, whereas Egerton et al., found differences in the ACC but not in the thalamus. It is unclear why the location of the differences in the two studies should be different. The respective remission and non-remission samples were comparable in size, and both studies used the same scanner and the same 1H-MRS protocol. However, the present study was restricted to patients that were minimally medicated or mediation-naïve at first presentation, and most of the patients were treated with the same antipsychotic drug (amisulpride) following a standardised dosing protocol. In contrast, the Egerton et al., study was open to any patient with a recent first episode of psychosis, regardless of what treatment they had been given. Thus, patients had been treated by clinical teams using a variety of different antipsychotics, with no restriction on type or dose. In the present study, antipsychotic use in the majority of patients was monitored for the first 5 weeks as part of a clinical trial, and so medication adherence may be higher in this sample compared to the Egerton et al. study. As the present sample is more homogenous in their medication exposure and duration of psychosis, this may increase the power to detect differences in the thalamus. Indeed, in the Egerton et al., study, the same pattern is present; Glx/Cr levels were lower in the remission group, although this did not reach significance.

Egerton et al., reported that high ACC glutamate levels correlated with a greater severity of negative symptoms, whereas in the present study, high thalamic Glx levels correlated with a greater severity of positive symptoms. Symptom scores at the time of scanning were similar between studies, except that non-remission patients had more severe negative symptoms in the Egerton study. This may explain why correlations with negative symptoms were not detected in the present sample. Furthermore, the Egerton study classified patients into remission and non-remission groups according to Andreasen's remission criteria (Andreasen et al., 2005) (see section 5.5.1 below for further discussion of the definition of response), whereas the present study classified patients according to the reduction in positive symptoms. This suggests that glutamate in the ACC may mediate negative aspects of disease, which is consistent with its role in emotional processing, whereas the thalamus is indicated in positive symptoms.

Differences in the way that the samples were ascertained and were treated also applies to the discrepancy between the findings from the present study and the two studies in chronic patients with treatment resistance (Demjaha et al., 2014; Mouchlianitis et al., 2015). A further consideration in relation to these studies is that the duration of antipsychotic treatment and of the disorder was much longer (16 and 14 years, respectively) in these chronic patients than in a first episode sample. Another study in chronic patients, following a minimum 7 day medication washout, and after 4 weeks of antipsychotic treatment, reported that baseline Glx/Cr levels in the frontal lobe, but not thalamus or medial temporal lobe, were higher in patients that did not go on to show more than a 20% reduction in total PANSS score (Szulc et al., 2013). However, these patients were temporarily medication-free rather than medication-naïve, and the extent to which the 'baseline' measures were affected by previous antipsychotic exposure is unclear.

5.2.3. Previous studies of the relationship between glutamate measures and symptom severity

A number of cross-sectional studies have examined the relationship between thalamic glutamate measures and either PANSS scores (Egerton et al., 2012; Yoo et al., 2009) or SANS and SAPS scores (Bustillo et al., 2010; Theberge et al., 2002) in patients with psychosis. None of these found significant correlations (see section 2.1 for a systematic review). One study differentiated patients based on symptom exacerbation; patients currently experiencing exacerbated psychotic symptoms had increased Glx levels in inferior parietal, but not frontal, white matter relative to both stable patients and healthy controls (Ota et al., 2012).

Only two previous studies have examined the association between longitudinal *changes* in glutamate measures and symptom severity. One study assessed antipsychotic-naïve FEP patients at presentation and following 4 weeks of treatment with risperidone. A reduction in glutamate and Glx levels in the associative striatum was correlated with an improvement in the PANSS general score (de la Fuente-Sandoval et al., 2013). The other study examined chronic patients who were medication-free for 6 months, although the majority were medication-naïve (Choe et al., 1996). A longitudinal reduction in prefrontal white matter Glx was correlated with a reduction in BPRS scores, following 4 weeks to 6 months of treatment with haloperidol, trifluoperazine, pimozide, or clozapine. Despite the differences in brain region, patient group and antipsychotic medication examined in these two studies, it is of interest that both reported that reductions in glutamate metabolites following antipsychotic treatment were correlated with symptomatic improvement. This is broadly

consistent with the findings of the present study, and suggests that clinically effective antipsychotic treatment is associated with a reduction in regional glutamate metabolite levels. This might explain why so few correlations between symptom scores and glutamate measures have been found in cross-sectional studies (see Chapter 2; Section 2.1 for systematic review, Section 2.2 for meta-regression, and Section 2.3 for multicentre analyses).

5.2.4. Previous studies examining the effect of medication on glutamate measures

Previous 1H-MRS studies examining glutamate measures before and after antipsychotic treatment did not classify patients according to response, and so the effects of treatment on thalamic glutamate measures may have been masked. One study found significantly lower thalamic glutamine levels after 30 months, but not 10 months of treatment, compared to baseline, when patients were antipsychotic-naïve (Theberge et al., 2007). The reduction in thalamic glutamine levels with antipsychotic treatment is consistent with my finding of reduced thalamic Glx levels after both 5 weeks and ~10 months of treatment, although it is of interest that glutamine reductions were not detected at 10 months, as the reduction in SAPS (Scale for the Assessment of Positive Symptoms) score indicate that the majority of patients were responders at this timepoint. Another study found no difference in thalamic Gln/Glu ratios in patients with schizophrenia relative to controls after 6 and 12 months of treatment (Bustillo et al., 2010).

Previous studies have not found evidence for reductions in glutamate metabolites in the ACC following antipsychotic treatment. In antipsychotic naïve/minimally treated FEP patients, ACC Gln/Glu ratio did not change after 6 and 12 months of treatment (Bustillo et al., 2010), and ACC glutamine levels did not change after 10 and 34 months of treatment (Theberge et al., 2007). In chronic patients, ACC Glx/Cr levels did not change following a switch from conventional antipsychotics to 8 weeks of treatment with olanzapine. However, in the latter study, when patients were split into responders and non-responders according to the change in negative symptoms, an increase in ACC Glx/Cr was seen in patients whose negative symptoms improved (Goff et al., 2002). This highlights the potential importance of defining patients according to therapeutic response, although this is in contrast with the results of the Egerton et al. study described above, as higher levels of ACC glutamate were associated with a greater severity of negative symptoms (Egerton et al., 2012).

Longitudinal reductions in glutamate levels in medication-naïve FEP patients have been reported in the striatum following 4 weeks of treatment with risperidone (de la Fuente-Sandoval et al., 2013). The patient sample was not subdivided according to response, but there was a 30% reduction in PANSS total score in the sample as a whole, suggesting that most patients were responsive. A preliminary study which did not specify the response of patients found reduced DLPFC glutamine in 8 FEP patients following ~14 weeks of antipsychotic treatment (Stanley et al., 1996).

5.3. Possible mechanisms

There is some evidence that psychotic disorders are associated with progressive brain changes (Hulshoff Pol and Kahn, 2008), although this remains controversial (Zipursky et al., 2013). Longitudinal reductions in thalamic Glx could therefore reflect changes linked to the natural history of the disorder, as opposed to an effect of treatment. However, this seems unlikely, as longitudinal reductions in Glx were not evident in the patient sample as a whole, but were limited to those that responded to treatment. Nevertheless, I cannot exclude the possibility that there is subtype of schizophrenia that has a poor prognosis, is unresponsive to antipsychotic treatment and is associated with persistently high Glx levels. However, if this were the case, one would expect it to be associated with high Glx levels prior to treatment.

If antipsychotic medication is responsible for longitudinal changes in thalamic glutamate function in patients that respond to treatment, what might be the mechanism? Antipsychotic treatment targets D2 receptors in the striatum to reduce the downstream effects of excessive dopamine release in this region. Efferents from the striatum project to the thalamus via the pallidum. In patients who respond to antipsychotics, the normalisation of the effects of excessive dopamine activity in the striatum may lead to a reduction in thalamic glutamate levels, via the cortico-striatal-midbrain-thalamic circuit, see Figure 32 (Alexander et al., 1986; Haber, 2003). In non-responders, striatal dopamine function may be relatively normal, and antipsychotics may therefore have little effect, allowing thalamic glutamate to remain elevated.

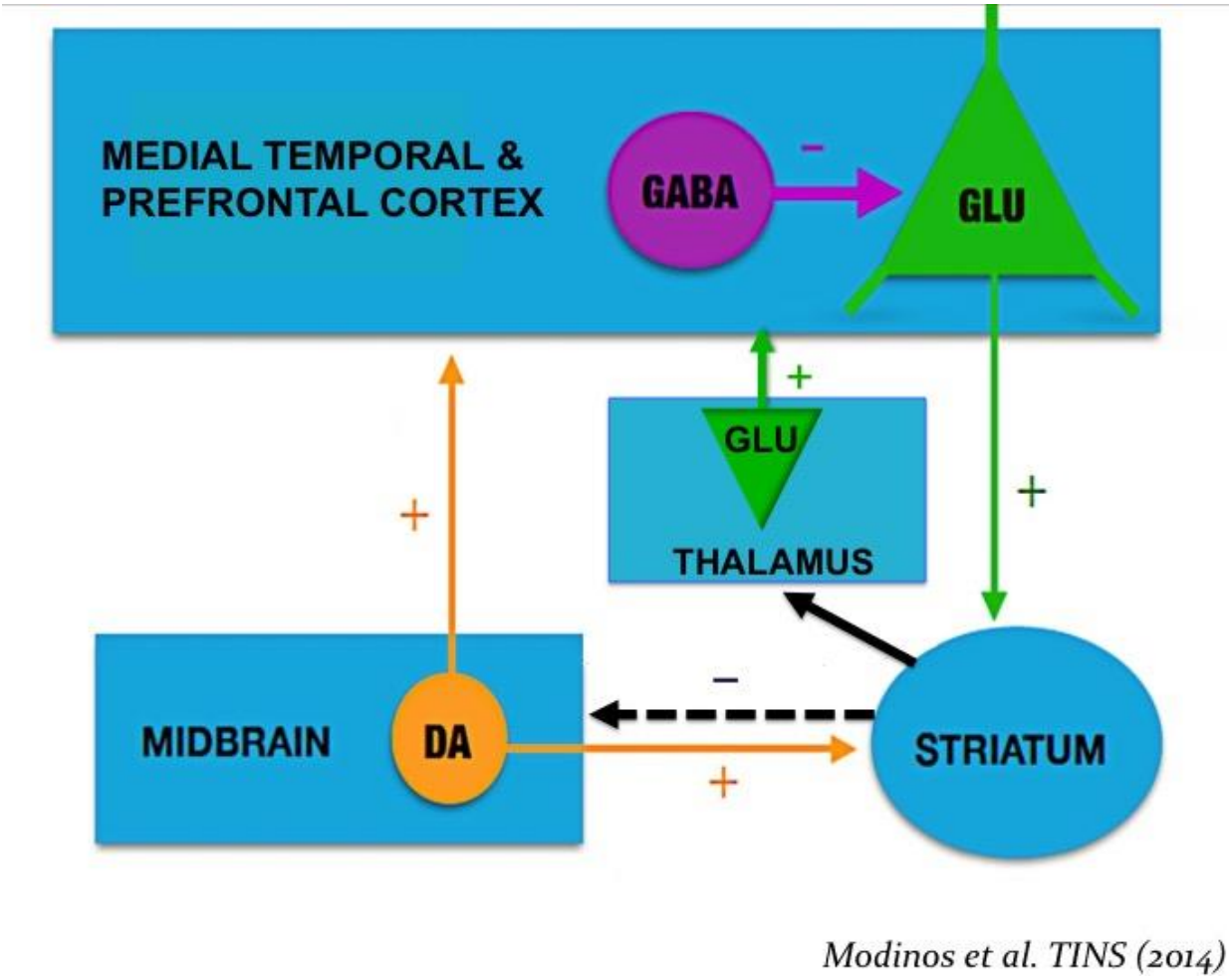

Figure 32 A model of the circuitry linking prefrontal glutamatergic activity to the control of striatal dopamine levels, which in turn feedback to the thalamus to affect glutamate levels in this region. Adapted from Modinos et al., TINS 2014.

The above findings, and the association between longitudinal changes in thalamic glutamate measures and symptomatic improvement suggest that pharmacological interventions aimed at reducing glutamate levels may help to alleviate symptoms in schizophrenia. However, to date, clinical trials of glutamatergic agents have shown only small to modest effects (see reviews Keefe et al., 2013; Papanastasiou et al., 2013; Singh and Singh, 2011). These disappointing results might reflect the involvement of chronic patients in all studies to date. My meta-analysis suggests that there are differences in the glutamatergic abnormalities in patients in the early and chronic stages of schizophrenia, and the present findings indicate that antipsychotic treatment has significant effects on these measures. Evaluating novel glutamatergic compounds in high risk or first episode patients, who have been minimally treated, may be more effective.

5.4. Prediction of psychosis

The present results are relevant to ongoing efforts to develop clinical tools that can be used to facilitate the prediction of treatment response in schizophrenia (Dazzan et al, 2015; McGuire et al, 2015). At present it is not possible to predict whether a given patient will or will not respond to antipsychotic medication. This can only be determined empirically, on the basis of a lengthy evaluation of the effectiveness of one or more courses of treatment. If brain glutamatergic measures differ in responders and non-responders, this suggests that they could be incorporated in to a predictive tool. The present findings suggest that repeated 1H-MRS scanning may be necessary to differentiate these subgroups, but further studies with larger patient samples are required to clarify whether a single baseline assessment might be sufficient. Using biological measures to stratify patients according to future response to antipsychotic medication would allow probable non-responders to be given alternative treatments, such as clozapine, at an earlier stage. At present, even in major clinical-academic centres, there is still a delay of around five years between patients being identified as treatment unresponsive and the initiation of treatment with clozapine (Howes et al., 2012b; McGuire et al., 2015).

1H-MRS glutamate measures may also be useful in clinical trials of novel therapeutic agents that are designed to act on the glutamatergic system. First, scanning patients before and after administration of a novel drug can be used to test whether it alters brain glutamate function (Egerton et al, 2016). 1H-MRS measurements may also reveal whether the response to the drug is related to glutamatergic measures at baseline and / or changes in these measures over the course of treatment. Finally, 1H-MRS glutamate scanning could also be used to identify subsets of patients with marked glutamatergic dysfunction: these subjects can then be used to form 'enriched' samples in whom a clinical effect of the drug may be more detectable.

5.5. Methodological considerations

5.5.1. Definition of response and remission

In the present study, the response to treatment was defined in terms of the reduction in PANSS positive symptom score, as opposed to the PANSS total symptom score, because antipsychotic medication mainly affects this symptom domain. In studies of antipsychotic treatment in patients with chronic schizophrenia, response is often defined as a reduction in symptoms of 20% or more (Leucht et al., 2007). However, because the symptomatic response is relatively good in first episode patients, a higher cut-off of 50% has been

recommended (Kahn et al., 2008; Leucht et al., 2007). I therefore used a 50% threshold in the present study, which yielded subgroups of approximately equal sizes (n=12 and n=9 at 5 weeks, and n=11 and n=10 at 10 months). The median reduction in positive symptoms in the sample was 60% at 5 weeks and 53% at 10 months. If a 20% threshold had been used, at 5 weeks most of the sample (n= 15) would have been responders, with only a minority (n= 6) non-responders, and at 10 months, 3 non-responders and 18 responders. This would have reduced the power to detect statistical differences between these subgroups.

An alternative approach to defining treatment response is to classify patients according to whether they are in symptomatic remission. The most widely used remission criteria are those defined by Andreasen et al (2005). These define remission in terms of ratings of mild or lower on 8 PANSS items that are thought to be specific for schizophrenia, and that the ratings are at these low levels for at least 6 months (Andreasen et al., 2005). The latter duration criterion could not be applied in the present study, as patients had not been regularly assessed for 6 months prior to the follow-up points. One potential advantage of using remission criteria as opposed to a percentage severity reduction criterion is that it avoids the possibility that a highly symptomatic patient could show a 50% reduction in symptoms yet still be symptomatic and, from a clinical perspective, unwell. However this does not appear to have been an issue in the present study: comparison of the subgroups defined using a 50% reduction in positive symptoms and by the remission criteria revealed that they largely comprised the same patients: after 5 weeks of treatment, 10 of 12 patients who show more than a 50% reduction in PANSS positive symptoms meet Andreasen criteria for remission, and at 10 months, 9 of 11 patients who show more than a 50% reduction in PANSS positive symptoms meet Andreasen criteria for remission.

5.5.2. Measurement of medication adherence

In the present study, the assessment of medication adherence relied on patient self-report and information in their medical records. Although this provides useful information on the extent to which patients were taking their treatment, there is a possibility that the differences between responders and non-responders were related to differing levels of adherence, rather than a difference in the effect of the medication. Recording pill dispensing and measuring blood antipsychotic levels could have provided a more accurate measure of adherence.

5.5.3. Limitations of 1H-MRS

The main findings in the present study involved measurements of Glx, as opposed to glutamate or glutamine. The data were acquired at a field strength of 3T and using a short echo time (30ms), and with these parameters, glutamate and glutamine signals cannot be completely resolved from each other, as their peaks overlap by <30% in the 2.25–2.55 ppm range (Snyder and Wilman, 2010a). Glx is therefore a more valid measure than glutamate and glutamine using the protocol that was employed in the study. Nevertheless, it is still notable that no differences in glutamate levels were detected, as glutamate makes up ~90% of the Glx signal. This could be because the differences in Glx were driven by changes in glutamine rather than glutamate. This would be consistent with the results of the meta-analysis, which indicated that patients with schizophrenia had higher thalamic glutamine but not glutamate levels (see Chapter 2). Further 1H-MRS studies using higher field strengths and that can quantify glutamine are needed to address this issue. However, at present, MRI scanners with field strengths of 4T or above are relatively uncommon.

CSF-corrected rather than creatine-scaled data were examined in the present study, as creatine scaled data relies on the assumption that creatine levels are stable in the brain and do not differ between clinical groups. In the present study, voxel CSF, grey and white matter content did not differ between clinical groups, and the main interaction findings in the thalamus remained significant when creatine-scaled data were assessed. This indicates that the main findings are robust and are not an artefact of variability in voxel CSF content. Furthermore, creatine levels did not differ between patients and controls or between clinical groups in the present study.

1H-MRS is unable to differentiate intracellular and extracellular glutamate metabolite measures, and thus the cellular location of Glx changes cannot be established. Furthermore, it is not known whether Glx differences between groups represent an alteration in glutamate used for metabolism or neurotransmission. Future studies able to measure glutamine at 4T will provide a more reliable indicator of glutamate neurotransmission, as the majority of glutamine is sourced from the metabolism of neurotransmitter glutamate. Many of these issues could be overcome by using PET or SPET ligands with a molecular level of specificity for elements of the glutamate neurotransmission system, such as the NMDA receptor. However, despite a great deal of research, there are still no PET or SPET tracers for glutamatergic receptors that have been validated. Pilowsky et al., (1993) reported plausible results in schizophrenia using a SPET

ligand, but it is not considered specific for the NMDAR. A number of candidate PET tracers for the NMDAR are currently being evaluated (McGinnity et al., 2015, 2014).

5.6. Future work

A causal association between antipsychotic treatment and a reduction in thalamic Glx levels cannot be shown by this study. To definitively determine whether the natural history of schizophrenia, as opposed to antipsychotic medication, mediates longitudinal changes in glutamatergic metabolites, would require a longitudinal 1H-MRS study in medication-naïve patients who remained medication-free. This would be challenging to conduct, as it would require the recruitment of first episode patients who either refused to take antipsychotic medication or in whom treatment was withheld. In most developed countries it would be regarded as unethical to withhold antipsychotic treatment, even if the patient refused treatment, especially if this was to be for a long period. An alternative method to decipher medication effects on glutamate would be to examine 1H-MRS measures longitudinally as patients discontinue medication.

Future 1H-MRS work using MRI scanners with a field strength of 4T or more would clarify the extent to which findings involving Glx reflected alterations in glutamate or glutamine (Theberge et al., 2007, 2002). The meta-analysis suggests that significant differences in glutamine, but not glutamate or Glx, are present in first episode patients compared to healthy controls in the ACC. Similarly in the thalamus, differences in glutamine, but not glutamate or Glx, are detected in cases in comparison to controls. Therefore further work at 4T may detect case-control differences which were not found in the present study.

The meta-analysis found elevations in glutamatergic metabolites in the medial temporal lobe and the basal ganglia of patients (Chapter 2.2), but no studies to date have investigated their relation to treatment response. It would therefore be of interest to examine glutamatergic concentrations in these regions, as the multicentre analysis reported a correlation between medial temporal Glx levels and negative symptoms (Chapter 2.3), and a recent study found that treatment normalised striatal glutamate levels in FEP patients (de la Fuente-Sandoval et al., 2013). In addition, abnormalities in GABA are thought to also play a role in schizophrenia, as elevated glutamate release is proposed to result from disinhibited GABAergic interneurons (Lisman et al., 2008). Future 1H-MRS studies using edited acquisition sequences that are able to measure GABA concentrations in the brain may further elucidate the pathways involved in schizophrenia aetiology.

The meta-analysis (Chapter 2.2) and the longitudinal study of 1H-MRS glutamate measures and their relation to treatment response (Chapter 4) conclude that schizophrenia is associated with elevations in glutamatergic metabolites, and that patients who respond to antipsychotic medication show a reduction in thalamic Glx levels whereas Glx levels do not change in treatment non-responders. These results indicate that novel compounds that reduce glutamatergic metabolites may be therapeutically beneficial in the non-responder group. Therefore future work should identify novel therapeutic compounds that reduce glutamate metabolite levels using 1H-MRS, and investigate their relationship to symptoms.

5.7. Conclusions

This thesis suggests that alterations in glutamatergic function are evident in a number of brain regions in schizophrenia, and that these differ between patients who do and do not respond to treatment with antipsychotic medication. The longitudinal 1H-MRS study indicates that in patients with first episode schizophrenia, a good response to treatment with drugs that block dopamine D2 receptors is associated with a longitudinal reduction in thalamic Glx levels. These findings have implications for our understanding of the pathophysiology of the disorder, the stratification of patients, and the development of novel treatments.

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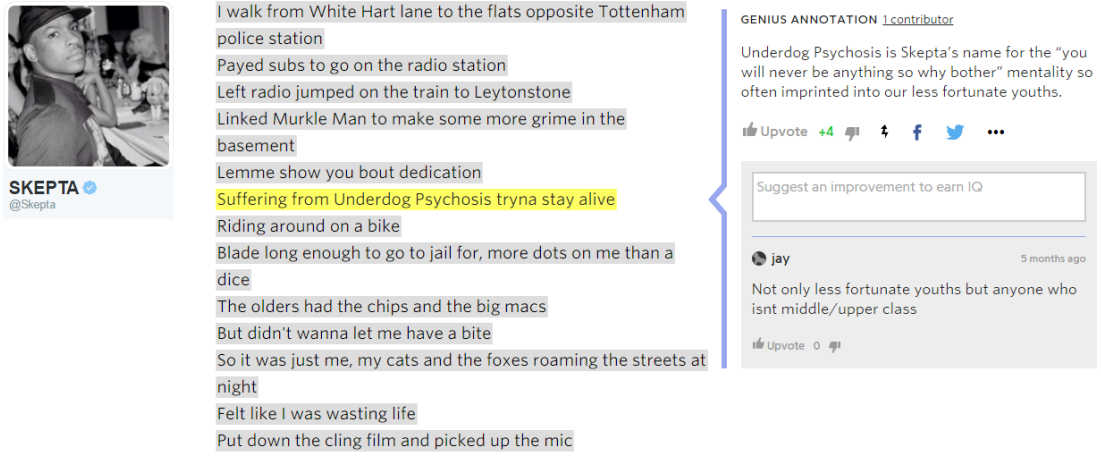

In this thesis I have discussed a neurochemical approach to schizophrenia. Schizophrenia however is a highly complex disorder, with no single causative gene or mechanistic pathway. I think it is important to consider the wider socioeconomic context of a mental health disorder after reducing it down to its molecular level. So, for the final page of my thesis, I would like to bring your attention to the lyrics of Skepta, a successful grime artist based in Tottenham, of Nigerian descent.

Skepta often refers to "underground psychosis" in his lyrics. This does not directly refer to psychosis, instead it refers to the vulnerable mental state that individuals enter into when a society does not believe they can provide anything of worth, and the vicious cycle that ensues where the individual gives up. I think we can learn something from Skepta's insight into the socioeconomic factors that increase the risk of schizophrenia, factors which may impact upon the physical pathways that have been discussed in this thesis.

Skepta himself does not suffer from a mental illness, however these lyrics do lead you to question what he may have encountered, had he not successfully transformed himself from a young black male disempowered by society into a pioneer of grime music in the UK in the early 2000s.